Immunohistochemical characterization of neo-tissues and tissue reactions to septal defect occlusion devices

Foth, Immunohistochemistry occlusion devices

Rudi Foth¹, Thomas Quentin¹, Ina Michel-Behnke MD², Manfred Vogt MD³, Thomas Kriebel MD¹, Anne Kreischer¹, Wolfgang Ruschewski MD⁴, Thomas Paul MD¹, Matthias Sigler MD¹

¹ Department of Pediatric Cardiology and Pediatric Intensive Care Medicine, Georg-August University, Goettingen, Germany
² Pediatric Heart Center, Justus-Liebig University, Giessen, Germany
³ Department of Pediatric Cardiology and Congenital Heart Disease, German Heart Center Munich at the Technical University, Munich, Germany
⁴ Department of Thoracic and Cardiovascular Surgery, Georg-August University, Goettingen, Germany

Corresponding author:
Matthias Sigler MD
Department of Pediatric Cardiology and Pediatric Intensive Care Medicine
Georg-August University Goettingen
Robert Koch Strasse 40
D 37099 Goettingen, Germany
Email: msigler@gwdg.de
Phone: ++49-551-396270
Fax: ++49-551-3922561

Total word count: 4604 words

Journal subject codes:

[41] Pediatric and congenital heart disease, including cardiovascular surgery
[162] Smooth muscle proliferation and differentiation
Abstract

Background – To evaluate tissue reactions within and at the surface of devices for interventional therapy of septal defects and to identify antigen characteristics of neo-tissues.

Methods and Results – Atrial septal defect or ventricular septal defect occlusion devices (Amplatzer n = 7; Cardioseal/Starflex n = 3) were processed using a uniform protocol after surgical removal from humans (implantation time 5 days to 4 years). Devices were fixed in formalin and embedded in methylmethacrylate. Serial sections were obtained by sectioning with a diamond cutter and grinding, thus saving the metal/tissue interface for histological evaluation. Immunohistochemical staining was performed using conventional protocols. Superficial endothelial cells stained positive for von Willebrand factor. Within the newly formed tissues, fibroblast-like cells were identified with a time dependent expression of smooth muscle cell maturation markers (smooth muscle actin, smooth muscle myosin, h-caldesmon, desmin) beside extracellular matrix components. Neo vascularization of the newly formed tissues was demonstrated with the typical immunohistochemical pattern of capillaries and small vessels. Inflammatory cells could be identified as macrophages (CD68 +) and both T- and B-type lymphocytes (CD3 +, CD79 +).

Conclusions – This is the first presentation of results from serial immunohistochemical staining of a collection of explanted human septal occlusion devices. A time dependent maturation pattern of the fibroblast-like cells in the neo-tissues around the implants could be described. Neoendothelialization was seen in all specimen with implantation times of 10 weeks or more. The time course of neoendothelialization as seen in our study further supports the clinical practice of anticoagulant or anti platelet therapy for 6 months following implantation. This time interval should be sufficient to prevent thromboembolic events due to thrombus formation at the foreign surface of cardiovascular implants.

278 words
Key words:

heart septal defects; immunohistochemistry; catheterization; occlusion; pathology
**Introduction**

Clinical interest and information in literature on transcatheter closure of septal defects are generally focussed on feasibility, safety and long-term clinical outcome. As the implants remain in the human heart for lifetime, biocompatibility has additionally emerged into the focus of interest ¹. Hitherto, there are few data on histopathology of explanted occlusion devices for atrial and ventricular septal defects ¹-⁴. Concerning immunohistochemical characterization of tissue reactions, data are scarce. Just one “clinical vignette” presenting one figure of an immunohistochemically labeled specimen has been published so far ⁵.

The purpose of this work was to characterize cellular and extracellular matrix (ECM) components that are formed within and around the devices after implantation and to illuminate the previously unknown way of ingrowth.

**Material and Methods**

**DEVICES**

The Amplatzer septal defect occluder (AGA Medical Corporation, Golden Valley, MN, USA) is a self-expanding device, made from Nitinol wire mesh. It has a double disc shape and consists of two retention disks with a short connecting cylindrical main body, into which polyester fibers are sewn. Devices for atrial septal defects and ventricular septal defects differ slightly in design.

The Cardioseal/Starflex device (NMT Medical Inc., Boston, MA) is constructed of a metal (MP35N) “double-umbrella” configured framework to which polyester fabric patches are attached on both sides. The only difference between the two implants is the addition of a non-ferromagnetic centering spring in the Starflex device.

**HUMAN EXPLANTS**
10 human tissue specimens were collected during corrective surgery at five centres and sent to us for routine histopathological work-up. The time intervals between implantation and explantation of septal defect occlusion devices ranged from 5 days to 48 months. Indications for device explantations are summarized in Table 1.

**Tissue Preparation**

Immediately after explantation, the tissue block containing the implant was dissected free with a minimum of surrounding tissue. After briefly flushing with saline, the specimen were fixed in formalin (buffered 4 %).

**Embedding, Sectioning, and Histology**

To obtain immunostaining of resin embedded specimen, the sections were mounted on glass slides using silicon glue and underwent deplastification as described previously. Staining with Richardson blue, Movat Pentachrome, or Picosirius red was performed according to standard protocols. For Richardson blue staining of resin embedded specimen, the sections were mounted on plastic slides and stained without deplastification. For Movat-Pentachrom and Picosirius-red staining the resin embedded specimen were mounted on glass slides and deplastificated as for immunostainings.

Prior to embedding, macroscopic evaluation and documentation was accomplished. After fixation, the tissue block with the device was embedded in resin methylmethacrylate (Technovit 9100, Kulzer & Co, Wehrheim, Germany). Following hardening, the resin blocks were subsequently sectioned in slices of 0.8 mm using a diamond band saw (300 CP, Exakt GmbH, Norderstedt, Germany). These slices were grinded down to 5-30 μm with a horizontal rotatory grinder and polisher (400 CS, Exakt GmbH, Norderstedt, Germany).

**Immunohistochemistry**

Details of the staining protocols are shown in table 2. Umbilical cord sections served as positive controls for immunostaining of smooth muscle markers and human tonsil sections for
immune cells respectively. Negative controls were processed without the antigen specific antibody.

NOMENCLATURE

In order to clarify the assignment of findings to different regions in the specimen, the following terms were used as shown in Figure 1:

- neo-endothelium for the superficial cell-layer - if present,
- pseudointima for the tissue in-between neo-endothelium and the implant, and
- neo-tissue within the implant for tissue inside fabric and metal wires of the implant.

The term pseudointima was employed according to previous descriptions of this type of tissue that is being formed intracardially after device implantation\textsuperscript{1,2}.

STATEMENT OF RESPONSIBILITY

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Presentation of results is focused on immunohistochemical labeling as well as results of Movat Pentachrome and Picrosirius red staining. Results of macroscopic evaluation and standard histology were – in part – presented and discussed previously by our group\textsuperscript{1}.

Neo-Endothelium

Macroscopically, the Amplatzer atrial septal defect occluder with an implantation time of only 5 days (patient 1) was covered with a thin layer of dull greyish material (Figure 2 a). By means of immunostaining, this superficial material could be identified as fibrin with included blood cells (Figure 2 b). All other devices with implantation times of $\geq 10$ weeks were covered by a shining layer of whitish tissue of variable thickness macroscopically (Figure 2 c). Endothelialization was complete except for some prominent parts of the occluder of
patient 2. Von Willebrand factor stained positive in all these specimen (Figure 2 d). Results of immunostainings are summarized in table 3.

**Fibrin deposition**

Formation of condensed fibrin with included blood cells around the foreign material was the principle finding in the specimen with an implantation time of 5 days (Figures 3 a). In the superficial portion of this fibrin deposit, single longitudinally oriented cells were seen. These cells stained positive for vimentin (Figure 3 b), but not for other muscle cell markers.

Residual islets of fibrin deposits were observed in single specimen with implantation times of up to 15 months (Figure 3 c). These fibrin residues were solely seen in the central portion of the implants. In all other specimen with implantation times of \( \geq 24 \) months, there was no evidence for thrombotic material within the implants.

**Pseudointima**

Except for the implant with an implantation time of 5 days, all specimen with an implantation time \( \geq 10 \) weeks had a well definable pseudointima with an organized cellular arrangement. The cells of the pseudointima showed a predominantly longitudinal orientation (parallel to the neo-endothelium; Figure 1 b) and stained positive for vimentin in all specimen (Figures 4 a). In addition, staining was positive for the muscle cell marker smooth muscle actin and smooth muscle myosin (Figures 4 b and c). H-Caldesmon as a marker of mature smooth muscle cells was detected in one specimen with an implantation time of 15 months (Figure 4 d). Desmin stained positive in two specimen with implantation times of 10 months and 48 months.

By means of Movat pentachrome stain, proteoglycans were identified as main component of ECM within the pseudointima. In addition, collagen and few elastic fibers were seen.

Immunohistochemically, antibodies against collagen III stained positive with a homogeneous distribution pattern.
Few capillaries and small vessels could be detected within the pseudointima. The cells lining the lumen of the capillaries stained positive with antibodies against von Willebrand factor and vimentin (Figure 5 a). In addition, cells in the wall of the small vessels showed positive staining for smooth muscle actin, smooth muscle myosin, and h-caldesmon even in specimen with implantation times of only 10 weeks (Figure 5 b).

**Neo-tissue within the implant**

The neo-tissue **within** the implant can be clearly distinguished from the formerly described pseudo-intima (a) by its localisation, (b) by the configuration of cellular and extracellular components, and (c) by its rich vascularisation opposed to the sporadic appearance of capillaries within the pseudointima. It morphologically consisted of areolar connective tissue with mostly irregular oriented cells (Figure 1 a). These cells stained positive with antibodies against vimentin, smooth muscle actin, and smooth muscle myosin (Figures 6 a and b).

Additional positive staining for desmin and h-caldesmon was found in single specimen with implantation times of more than 10 months.

As described above for the pseudointima, capillaries within the implant stained positive for von Willebrand factor, vimentin, smooth muscle actin, smooth muscle myosin, and h-caldesmon (Figures 5 a and 5 b).

ECM components were analyzed by means of movat pentachrome stain as well as immunohistochemical labeling with antibodies against collagen III (Figure 6 c).

Proteoglycans were identified as the main constituent of this part of the specimen besides collagen and few elastic fibers (Figure 6 d). Composition of ECM components was not different in the parts adjacent to dacron fibers and metal parts of the implants.

**Inflammation**

Foreign body giant cells were seen usually in local relation to dacron fibers. These cells stained positive with antibodies against vimentin as well as CD68 (Figure 7 a). In almost all
explants, inflammatory infiltrates with lymphocytes were observed within the implant. In two of the implants, marker for T- and B-cells were employed for further differentiation. In both specimen, lymphocytes stained positive with antibodies against the T-cell marker CD3 as well as with the B-cell marker CD79 (Figures 7 b and c).

The clinically most important aspects of the healing response (neo-endothelialization and cellular organization of initially formed fibrin deposits) after implantation of a septal defect occlusion device are summarized in table 4.

Discussion

To the best of our knowledge this is the first study that characterizes tissue reactions in a series of human explanted septal defect occlusion devices by means of immunohistochemistry. Two of the currently most commonly used septal occluder, the Amplatzer and Cardioseal/Starflex device, were evaluated in our study.

Except for one case report, no immunohistochemical stainings of explanted septal defect occlusion devices have been published so far 5. This may be for the most part due to the technical challenge to perform immunohistochemistry in hard resin embedded specimen 7,8. The need for deplastification of the grinded sections in order to uncover antigens aggravates the complexity of specimen processing 8. In addition, establishment of primary antibodies in resin embedded specimen requires far more time and effort as compared to paraffin wax embedded specimen.

Neoendothelialization of septal defect occlusion devices after interventional application is of major clinical relevance since superficial thrombus formation is a possible source for embolism and subsequent organ damage 9. For prevention, most centers give anti platelet or anticoagulant therapy for 6 months until neoendothelialization is exspected to be completed.
In animal studies and human explants endothelial cells were observed as soon as 30 days after implantation. In previous publications, these characterizations were accomplished solely by standard stain or scanning electron microscopy. In this study we were able to identify endothelial cells by means of immunohistochemical labeling for the first time. Antibodies against von Willebrand factor and vimentin stained positive superficial cells in all specimens with implantation times ≥ 10 weeks. Thus we could demonstrate functional properties in addition to formerly described morphological characteristics of endothelium on the intracardial surface of the implants.

After implantation of septal defect occlusion devices, initial formation of thrombotic material in-between metal wires and around polyester fibers has been described. This thrombotic material has been demonstrated to be transformed to connective tissue consisting of mainly ECM and embedded cellular components which morphologically resemble fibroblast-like cells in septal defect occlusion devices and occlusion devices for other applications. The results of our present study give some insight into the time course of this process as cellular organization of fibrin deposits was advanced after 10 weeks already, but completed after 24 months only (table 4).

To further characterize the “fibroblast-like” cells within the newly formed tissue we employed a panel of muscular maturation marker. The cells seen in the pseudointima as well as within the implant concordantly stained positive for the mesenchymal marker vimentin and the early muscular differentiation marker smooth muscle actin and smooth muscle myosin resembling a myofibroblast-like phenotype. H-caldesmon is part of the contractile apparatus of muscle cells and is regarded as a marker for mature and highly differentiated muscle cells. In our series, it only stained positive in one specimen with an implantation times of 15 months. Likewise, desmin as marker for intermediate filaments is expressed in highly differentiated muscle cells. The late occurrence of desmin and h-caldesmon in our specimen may be
taken as sign for the slow progression of cell maturation after device implantation.

Concerning immunostaining, there were no differences between cells in the pseudointima (longitudinally orientated) and cells within the implant (irregularly orientated).

Cells in close local relation to metal struts stained positive for vimentin, smooth muscle actin, and smooth muscle myosin independently of the implantation time. Thus we found no evidence for a possible toxic reaction to metal ions, as release of nickel ions from metal wires of septal defect occlusion devices is a well described phenomenon\(^\text{15,16}\).

In analysing the newly formed tissues around occlusion devices, the specimen with the shortest implantation time of only 5 days is of special interest. Besides the typical pattern of thrombus with fibrin septations and included blood cells, occurrence of some vimentin positive fibroblast-like cells could be demonstrated. These fibroblast-like cells could be fibrocytes. Fibrocytes are circulating cells, which migrate into wounds and adopt a smooth muscle like phenotype (spindle shape morphology, positive staining for vimentin and smooth muscle actin). Varcoe et al\(^\text{17}\) could demonstrate that these cells are involved in intima formation. It can be assumed, that these cells are involved in the organization of the thrombotic material and the formation of the pseudointima as seen in our collection of specimen.

With regard to ECM components, we found a similar areolar-like pattern in central parts of the specimen as described by Kreutzer with mainly proteoglycans besides collagen and few elastic fibers\(^2\).

Multinucleated foreign body cells have constantly been observed in local relation to dacron fibers of cardiovascular implants such as the Amplatzer or the Cardiosceal/Starflex device\(^2,4\). Foreign body giant cells in the specimen of our study stained positive with antibodies against CD68 identifying these cells as macrophages, which are considered as being part of the innate immune response.
In addition to the foreign body reaction, mild lymphocytic infiltrations have been described within the newly formed tissue after implantation of septal defect occlusion devices \(^1\)^\(^2\). In the present study, we were able to further differentiate this lymphocytic reaction for the first time, as we could demonstrate a lymphocytic reaction of a mixed type (CD3 and CD 79 positive). Thus we have evidence of some kind of adaptive cellular response in addition to the reaction of the innate immune system locally related to the implants.

No difference in pattern or time course of immunohistochemistry-based findings was observed comparing Amplatzer and Cardioseal/Starflex occlusion devices, different sites of implantation (ASD vs. VSD), or indication for explantation. This is in accordance with results of comparative analysis of standard staining of the two devices, as it has been reported earlier by our group \(^1\).

In summary, this study demonstrates the value of immunohistochemical staining for characterization of tissues within and around septal defect occlusion devices after interventional implantation. We can for the first time describe antigen patterns of cells in the neo-tissues. Fibroblast-like cells were identified with a slowly progressing maturation pattern over time as demonstrated by immunohistochemically labeling with a variety of smooth muscle cell markers. Superficial cells could be identified as endothelial cells. The time course of neoendothelialization as seen in our study further supports that clinical practice of anticoagulant or anti platelet therapy for 6 months following implantation. This time interval should be sufficient to prevent thromboembolic events due to thrombus formation at the foreign material surface of cardiovasular implants.

**Acknowledgements**

The authors thank Andrea Poppe and Karin Baer for technical assistance.
Disclosures

We have no conflict of interests.
References


Figure Legends

**Figure 1:** Micrographs show representative staining with Richardson blue (cellular components, blue; metal wires, black). Overview of an Amplatzer atrial septal defect occlusion device 24 months after implantation (patient 7). 1 A (detail), representative image of pattern of neo-tissue within the implant with irregular orientation of cells. 1 B (detail), representative image of pattern of cells of pseudointima with longitudinal orientation (parallel to the neo-endothelium).

**Figure 2:** 2 A, macroscopic aspect of an Amplatzer atrial septal defect occluder with an implantation time of 5 days (patient 1). 2 B, corresponding immunohistochemical staining with fibrin adjacent to Dacron fibres (DF; positive immunohistochemical staining, brown). 2 C, macroscopic aspect of an Amplatzer atrial septal defect occluder with an implantation time of 15 months (patient 6). 2 D, corresponding immunohistochemical staining with von Willebrand factor demonstrating positive labeling of neo-endothelium (positive immunohistochemical staining, brown; metal wire, black).

**Figure 3:** Micrographs show representative immunohistochemical staining of fibrin deposits (positive immunohistochemical staining, brown; metal wires, black; Dacron fibers, DF). 3 A, fibrin staining of a specimen with an implantation time of 5 days (patient 1). 3 B, micrograph of vimentin staining in the same patient. 3 C, micrograph of fibrin staining in patient 6 (implantation time 15 months).

**Figure 4:** Micrographs show representative immunohistochemical staining of cellular components of the pseudointima with typical longitudinal orientation of cells and few
capillarization (positive immunohistochemical staining, brown; Dacron fibers, DF). 4 A, micrograph of vimentin staining of a specimen with an implantation time of 48 months (patient 10). 4 B, micrograph of smooth muscle actin staining of a specimen with an implantation time of 12 months (patient 5). 4 C, micrograph of smooth muscle myosin staining of a specimen with an implantation time of 12 months (patient 5). 4 D, micrograph of h-caldesmon staining of a specimen with an implantation time of 15 months (patient 6).

Figure 5: Micrographs show representative immunohistochemical staining of capillaries and small vessels (positive immunohistochemical staining, brown; metal wires, black; Pseudointima, PI; Dacron fibers, DF). 5 A, micrograph of von Willebrand factor staining of a specimen with an implantation time of 10 weeks (patient 2). 5 B, micrograph of smooth muscle actin staining of a specimen with an implantation time of 48 months (patient 10).

Figure 6: Micrographs show representative immunohistochemical staining or Movat pentachrom staining of cellular and ECM components of tissue within the implant with typical irregular orientation of cells (positive immunohistochemical staining, brown; Dacron fibers, DF). 6 A, micrograph of vimentin staining of a specimen with an implantation time of 10 months (patient 4). 6 B, micrograph of smooth muscle actin staining of a specimen with an implantation time of 15 months (patient 6). 6 C, micrograph of collagen III staining of a specimen with an implantation time of 48 months (patient 10). 6 D, micrograph of Movat pentachrom staining (proteoglycans, bluish green; cell nuclei, red; collagen, yellow) of a specimen with an implantation time of 48 months (patient 10).

Figure 7: Micrographs show representative immunohistochemical staining of inflammatory cells (positive immunohistochemical staining, brown; Dacron fibers, DF). 7 A, micrograph of
CD68 staining labeling foreign body giant cells adjacent to Dacron fibers in a specimen with an implantation time of 36 months (patient 9). 7 B, micrograph of CD3 staining with positive labeling of T-lymphocytes in a specimen with an implantation time of 48 months (patient 10). 7 C, micrograph of CD79 staining with positive labeling of B-lymphocytes in a specimen with an implantation time of 15 months (patient 6). Details of 7 B and 7 C show positive controls in methylmethacrylate embedded human tonsil.
**Tables**

**Table 1:** List of specimens for histopathological work-up

<table>
<thead>
<tr>
<th>Patient</th>
<th>Implant</th>
<th>Implantation time</th>
<th>Indication for explantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amplatzer ASD</td>
<td>5 days</td>
<td>Malpositioning</td>
</tr>
<tr>
<td>2</td>
<td>Amplatzer VSD</td>
<td>10 weeks</td>
<td>Valve incompetence</td>
</tr>
<tr>
<td>3</td>
<td>Amplatzer VSD</td>
<td>7 months</td>
<td>Malpositioning</td>
</tr>
<tr>
<td>4</td>
<td>Amplatzer VSD</td>
<td>10 months</td>
<td>Malpositioning</td>
</tr>
<tr>
<td>5</td>
<td>Amplatzer ASD</td>
<td>12 months</td>
<td>Malpositioning</td>
</tr>
<tr>
<td>6</td>
<td>Amplatzer ASD</td>
<td>15 months</td>
<td>Residual Shunt</td>
</tr>
<tr>
<td>7</td>
<td>Amplatzer ASD</td>
<td>24 months</td>
<td>Residual shunt</td>
</tr>
<tr>
<td>8</td>
<td>Cardioseal ASD</td>
<td>25 months</td>
<td>Residual shunt</td>
</tr>
<tr>
<td>9</td>
<td>Cardioseal ASD</td>
<td>36 months</td>
<td>Atypical configuration of the device</td>
</tr>
<tr>
<td>10</td>
<td>Starflex ASD</td>
<td>48 months</td>
<td>Recurrent neurology/apoplexy</td>
</tr>
</tbody>
</table>

ASD – atrial septal defect; VSD – ventricular septal defect
<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Dilution</th>
<th>Antigen Retrieval</th>
<th>Secondary Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse anti-human desmin, clone D9 (Progen Biotechnik GmbH, Heidelberg, Germany)</td>
<td>1:50</td>
<td>Basic buffer, pH 9 (Target Retrieval Solution, High pH, Dako), 20 min in a steamer</td>
<td>Rabbit anti-mouse immunoglobulin antibody (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Mouse anti-human smooth muscle myosin heavy chain, clone ID8 (Chemico International, Temicon, Ca, USA)</td>
<td>Paraffine 1:100, Resin 1:500</td>
<td>Citrate buffer, pH 6 (Dako REAL™ Target Retrieval Solution), 40 min in a steamer</td>
<td>EnVision amplification system</td>
</tr>
<tr>
<td>Mouse anti-vimentin, clone V9 (Dako)</td>
<td>1:100</td>
<td>Citrate buffer, pH 6, 40 min in a steamer</td>
<td>EnVision amplification system</td>
</tr>
<tr>
<td>Mouse anti-human smooth muscle actin, clone 1A4 (Dako)</td>
<td>1:50</td>
<td>Citrate buffer, pH 6, 40 min in a steamer</td>
<td>Rabbit anti-mouse immunoglobulin antibody (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Polyclonal rabbit anti-human von Willebrand factor (Dako)</td>
<td>1:400</td>
<td>Citrate buffer, pH 6, 40 min in a steamer</td>
<td>Swine anti-rabbit immunoglobulin antibody (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Mouse anti-human caldesmon, clone h-CD (Dako)</td>
<td>Paraffine 1:100, Resin 1:400</td>
<td>Protease K (Dako, diluted 1:500 in 50 mM TBS, pH 7.6) at 37 °C for 5 min, followed by citrate buffer, pH 6 for 40 min in a steamer</td>
<td>EnVision amplification system</td>
</tr>
<tr>
<td>Polyclonal rabbit anti-human-CD3 (Dako)</td>
<td>1:100</td>
<td>Citrat ph 6 (Dako), 40 min in a steamer</td>
<td>Polyclonal rabbit anti-goat immunoglobulin/horsadishperoxidase (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Monoclonal mouse anti-human CD79αcy, clone HM57 (Dako)</td>
<td>1:100</td>
<td>Tris/EDTA, ph 9, 20 min in a steamer</td>
<td>Rabbit anti-mouse immunoglobulin (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Monoclonal mouse anti-human CD68, clone PG-M1 (Dako)</td>
<td>1:200</td>
<td>Tris/EDTA, ph 9, 20 min in a steamer</td>
<td>Rabbit anti-mouse immunoglobulin (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Monoclonal mouse anti-collagen III (Acris)</td>
<td>1:500</td>
<td>Citrat buffer, ph 6, 40 min in a steamer</td>
<td>Rabbit anti-mouse immunoglobulin antibody (Dako), diluted 1:100</td>
</tr>
<tr>
<td>Monoclonal mouse anti fibrin (No.350) (American Diagnostica inc. Stamford, CT, USA)</td>
<td>1:500</td>
<td>Citrat buffer, ph 6, 40 min in a steamer</td>
<td>Rabbit anti-mouse immunoglobulin antibody (Dako), diluted 1:100</td>
</tr>
</tbody>
</table>
Table 3: Staining patterns of primary antibodies

<table>
<thead>
<tr>
<th>Neo tissue</th>
<th>Stainings</th>
<th>Septal defect occlusion devices (Implantation time)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface</td>
<td>Amplatzer ASD (5 days)</td>
</tr>
<tr>
<td>Surface</td>
<td>vWF</td>
<td>-</td>
</tr>
<tr>
<td>Pseudo-intima</td>
<td>Vimentin</td>
<td>+++</td>
</tr>
<tr>
<td>SMM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>h-Cald</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Desmin</td>
<td>-</td>
<td>n. d.</td>
</tr>
<tr>
<td>Tissue within the implant</td>
<td>Vimentin</td>
<td>+++</td>
</tr>
<tr>
<td>h-Cald</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Desmin</td>
<td>-</td>
<td>n. d.</td>
</tr>
</tbody>
</table>

ASD – atrial septal defect; VSD – ventricular septal defect; vWF – von Willebrand factor; SMA – smooth muscle actin; SMM – smooth muscle myosin; h-Cald – h-Caldesmon
- negative; + positive; ++ strong positive
**Table 4:** Healing response over time

<table>
<thead>
<tr>
<th>Localization</th>
<th>Implantation time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days</td>
</tr>
<tr>
<td><strong>Surface</strong></td>
<td>Fibrin</td>
</tr>
<tr>
<td><strong>Pseudointima</strong></td>
<td>Fibrin/cells</td>
</tr>
<tr>
<td><strong>Tissue within the implant</strong></td>
<td>Fibrin</td>
</tr>
</tbody>
</table>
Immunohistochemical characterization of neo-tissues and tissue reactions to septal defect occlusion devices

Rudi Foth, Thomas Quentin, Ina Michel-Behnke, Manfred Vogt, Thomas Kriebel, Anne Kreischer, Wolfgang Ruschewski, Thomas Paul and Matthias Sigler

Circ Cardiovasc Interv. published online February 20, 2009;
Circulation: Cardiovascular Interventions is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-7640. Online ISSN: 1941-7632

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circinterventions.ahajournals.org/content/early/2009/02/20/CIRCINTERVENTIONS.108.810507

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Interventions can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Interventions is online at:
http://circinterventions.ahajournals.org/subscriptions/