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# Microscopic and histological analysis of the processes occurring in the aperture and wall of a coronary vessel after stent implantation

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The serious problem of angioplasty is restenosis, which relates to approx. 15–30% of all the patients subjected to the procedure. The present research was inspired by an attempt to explain this phenomenon and to analyse its causes. Two coronary arteries coming from the patients after stent implantation were subjected to analysis using light and electron microscopy. As a result of expansion of the stent and pressing the implant metallic structure into the artery wall, it comes to breaking the endothelium continuity, uncovering the structures of the intercellular matrix and the internal membrane and, consequently, to creation of a thrombus in the damaged area. As a result of the tissue response, the extracellular matrix is created and neointime formed.

*Key words*: *stent implantation, restenosis, vessel damage, angioplasty*

## 1. Introduction

The diseases of a cardiovascular system come to the fore in the statistics of diseases in the contemporary society. They constitute the main cause of sudden deaths of 40% of middle-aged men, and among women are the second cause of premature deaths after tumours. Nowadays in Poland, almost one million Poles suffer from the heart ischemic disease which results in approx. 90 thousand deaths a year [1].

There are many ways of treating the heart ischemic disease. One of them is the coronary angioplasty, which consists in restoring the lumen of a narrowed artery using a balloon-tipped catheter, introduced into the narrowed place. The first successful procedure was carried out in 1977 in Zurich and it initiated a new era in treating the heart ischemic disease [2], [3]. However, some possible postoperative complications related to delamination of the artery wall and repeated stenosis made the researchers seek the methods to improve the results and safety of the procedure. To that end, short metallic cylinders, called stents, were introduced, which, after implementation in the coronary vessel wall, make a scaffolding that allows us to maintain patency and prevents elastic rebounding of an atherosclerotic plaque. Numerous studies on animal models and clinical tests of those implants demonstrated significant improvement of the angioplasty results [4]–[9].

Nowadays, on the market there are many types of stents that differ from one another in the technique of expansion in the narrowed coronary vessel, shape, manufacturing technology and material [10]. They have characteristic biomechanical properties that assure safety, high repeatability and maximum efficiency of the procedure. Nevertheless, the still unsolved problem of coronary angioplasty is a recurrence of the stenosis, called restenosis.

Restenosis, as demonstrated in an angiographic examination, means reduction of the vessel diameter

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by at least 50% with respect to the values obtained as a result of coronary artery dilatation. This phenomenon concerns a relatively large population of patients, i.e., approx. 15–30% of all the patients subjected to the stent implantation [6]. This is caused by numerous cellular and molecular mechanisms that occur in the vessel lumen and wall as a reaction to mechanical damage to the vessel wall [2], [6], [11]–[15].

Three stages of the process of lesions in the vessel after implantation can be distinguished [11], [15]:

- early response to the damage caused by mechanical expansion of the coronary stent; it comes to a thrombus formation and to quick reaction of the expanded vessel;
- degranulation of the leukocyte cells with stimulation of fibroblasts and mioblasts to proliferation which leads to the enlargement of intercellular matrix being the main cause of neointime formation;
- late stage related to the rebuilding of the vessel wall that leads to closing the vessel lumen.

Research into restenosis has lead to defining a series of factors that can contribute to its development. These factors can be subdivided into clinical and anatomical ones. The clinical factors include unstable angina pectoris, male sex, diabetes, hypercholesterolemia, kidney insufficiency and smoking. The anatomical factors include high stenosis degree before the procedure, calcinosis in the atherosclerotic plaque to be expanded, long narrowed sections, small vessel diameter and stenosis at the vessel embranchment [2], [11], [15].

## 2. Subject and methodology of examination

The present research was aimed at microscopic and histological examination of the mechanisms occurring in the vascular lumen and wall, resulting in restenotic lesions in the place where the vessel was extended with a coronary stent.

Two coronary arteries were subjected to the examination, coming from the patients after angioplasty procedure using stents. In both cases, the implants of the same type were applied.

The artery No. 1 came from a 49-year-old man and was taken 3 days after the implantation of the coronary stent. The artery No. 2 came from a 52-year-old man and was taken 3 years after the implantation. The macroscopic examination revealed significant reduction of the vessel lumen at the place expanded with the stent as well as calcinoses in the expanded atherosclerotic plaque (see figure 1).

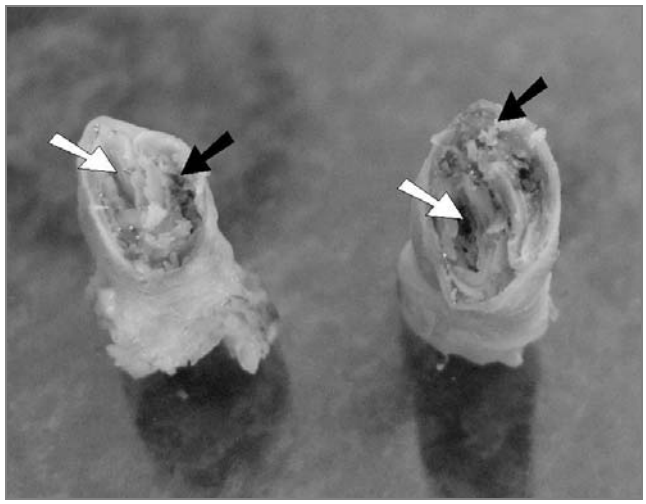

Fig. 1. Coronary artery No. 2 – three years after the implantation. Visible reduction of the vessel lumen in the expanded section (white arrow) and calcium deposits in the expanded atherosclerotic plaque (black arrow)

The material for histological and microscopic examinations was preserved in 2.5% and 5% solutions of glutaric aldehyde (to 0.1 M) and phosphate buffer of pH from 7.2 to 7.4. The samples were then washed, dehydrated in alcohol series and sealed in paraffin. 5-μm thick snips cut out of the artery cross-section were dyed with hematoxylin and eosine. The histological preparations obtained in this way were examined under a light microscope at magnification from 40× to 400×.

After the histological examination the material was deparaffined in several subsequent baths in xylene. After drying, the material was sprayed with an amorphous layer of gold. The analysis was performed using a scanning electron microscope JEOL 5800LV (SEM) at 150× to 30000×. The chemical composition of restenotic lesions was determined using an X-ray microanalyser Oxford Link 300, which is an integral part of the scanning microscope.

## 3. Results

### Artery No. 1

During the expansion of the stent No. 1 inside the coronary vessel, the continuity of the endothelium was broken, sub-endothelium matrix and internal membrane collagen uncovered, flexible fibres were intensely stretched and – in extreme cases – even

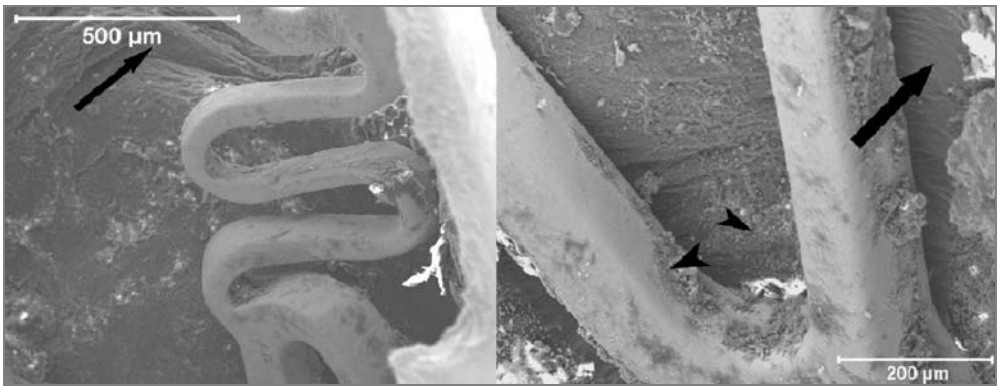


Fig. 2. Inside wall of the coronary vessel after the implantation. Visible damages to the endothelium caused by strong pressure of the implant metallic structure (arrow). SEM

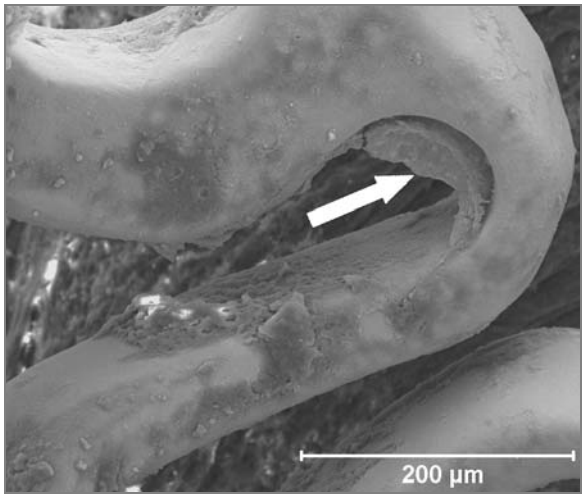


Fig. 3. A fragment of the implanted coronary stent with visible subepithelial tissue damaged by friction forces. SEM

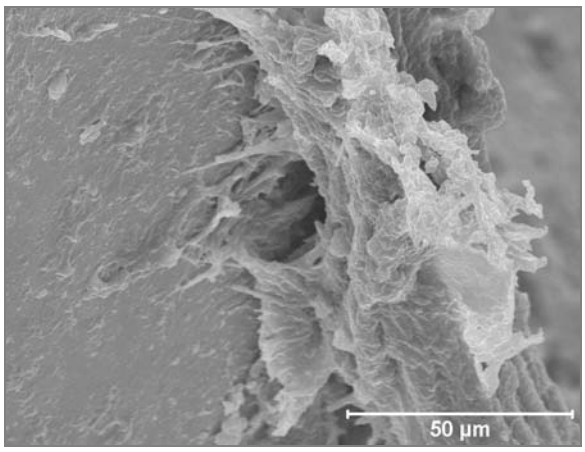


Fig. 4. Thrombus formed as a result of the endothelium response to the damage during the implantation. SEM

broken (see figures 2, 3). The damage resulted from the strong pressing of the implant metallic structure into the vessel wall and from additional friction forces between them.

The breaking of the endothelium continuity results in the discrimination of antithrombotic functions caused by creation of nitrogen oxide and prostacycline, required for correct blood flow in the vessel [1].

In addition, uncovering the connective tissue fibres and the intercellular membrane is conducive to adhesion and activation of thrombocytes and bonding of fibrinogen, which finally has lead to a thrombus in the expanded vessel area (see figure 4). The formation of a thrombus can increase the risk of infarcts.

### Artery No. 2

As a result of the vessel damage during the angioplasty procedure, the extracellular matrix was created and neointime formed, being the main cause of the secondary reduction of the artery No. 2 lumen (see figure 5). In addition, the calcinoses observed in the expanded atherosclerotic plaque are one of the factors that can favour development of the stenosis (see figures 6, 7).

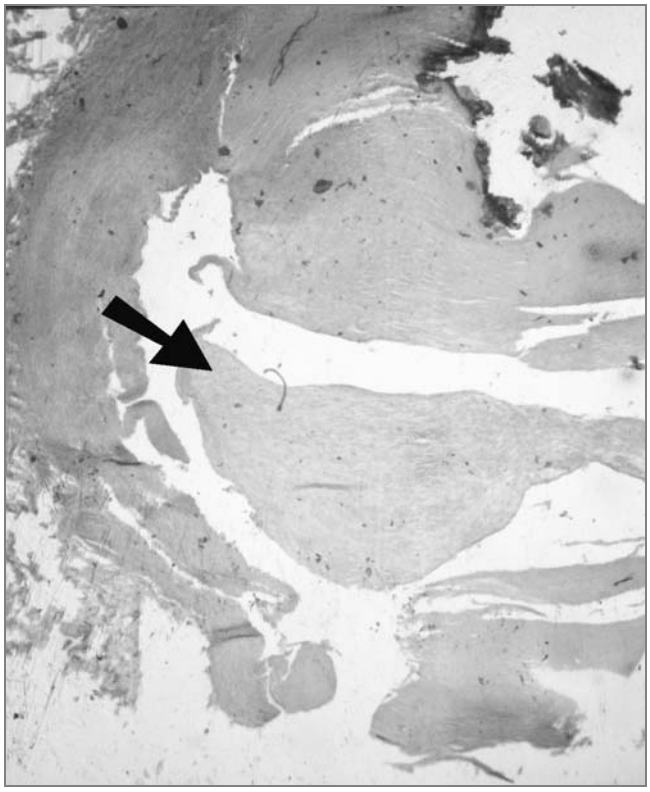

Fig. 5. Cross-section through the coronary artery No. 2. Visible reduction of the vessel lumen and neointimal tissue (arrow). Magnification of 40×, light microscope

Formation of neointime is attributed to migration of the inside membrane of smooth muscle and adventitia cells, activated during the vessel damage. The analysis of the histological preparations revealed an irregular pattern of flexible and collagen fibres in the neighbourhood of the hole made due to removing the stent (see figure 8). However, it is difficult to confirm the cell migration entirely. The features of the fibres observed prove pressing the fragments of the implant metallic structure into the vessel wall. This probably happens at the stage of heart muscle systole, which results in the outside pressure on the coronary arteries [16], [17].

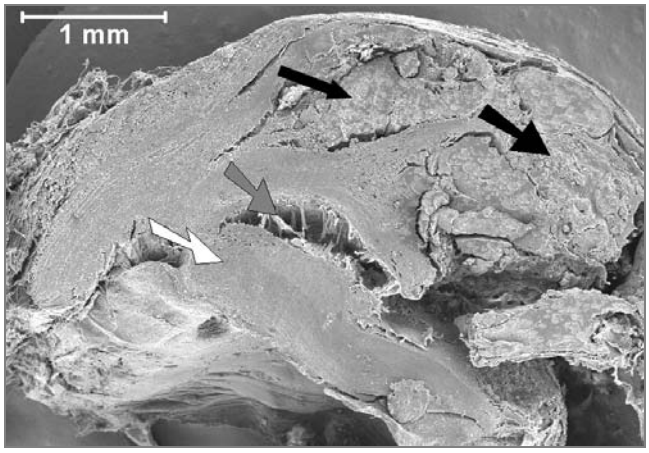


Fig. 6. A fragment of the coronary artery No. 2 with restenotic lesions (white arrow) and calcium deposits (black arrow) in the expanded atherosclerotic plaque. Gap after the stent removal is visible in the middle of the specimen (grey arrow) SEM

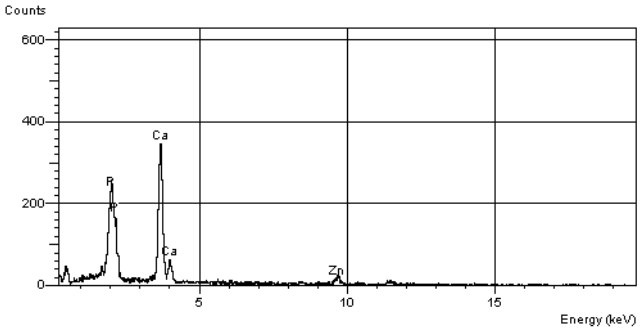


Fig. 7. X-ray energy spectrum representing the expanded atherosclerotic plaque that confirms the presence of calcium

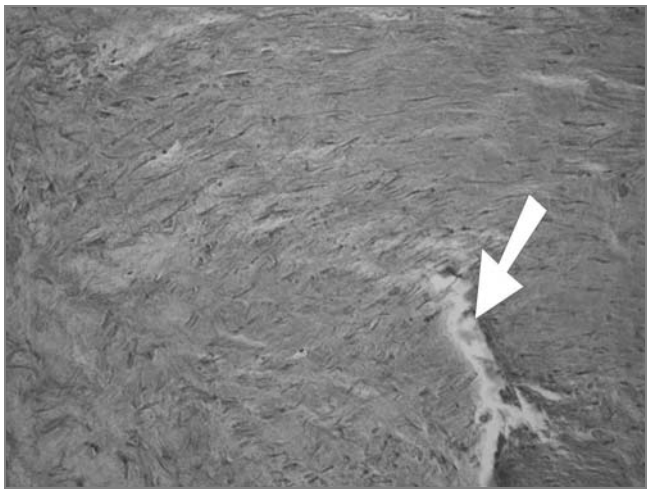

Fig. 8. Irregular pattern of fibers and accompanying cells at the hole (arrow) left after removal of the implanted stent (arrow). Magnification of 200× (11) and 400× (12), light microscope

Inflammatory process can have its origin in a heavy damage to the coronary vessel by the stent (see figure 9). In the histological preparations examined, in deeper layers of the vessel and in the places damaged by pressing implant fragments, neutrocytes were observed. These cells have the ability to take part in phagocytosis and numerous hydrolytic enzymes released by them can initiate the inflammatory process.

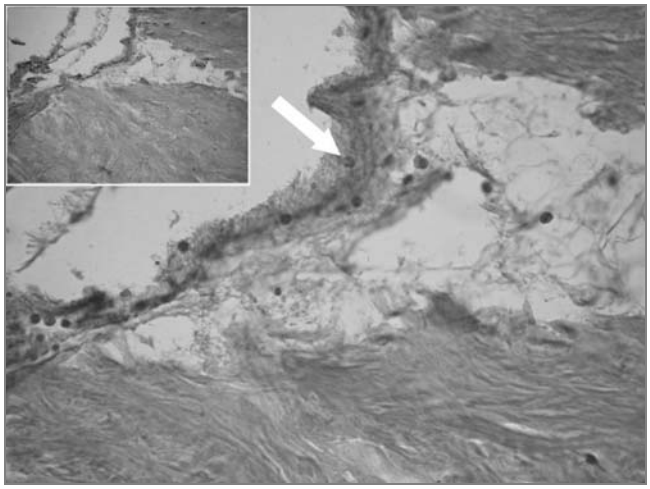

Fig. 9. Neutrophiles observed in deeper layers of the artery No. 2 walls. Magnification of 200× and 400×, light microscope

# 4. Conclusions

One of the most important achievements of the last years in the field of interventional cardiology directed to the treatment of the heart ischemic disease was the introduction of intravessel implants, called stents. However, in spite of such a great progress, acute and sub-acute closure of a coronary artery and recurrent stenosis, called restenosis, still remain unsolved problems. An attempt to explain this phenomenon and to find its causes inspired the present research.

Expansion of the coronary stent inside the artery results in the heavy damage to the endothelium, uncovering the sub-endothelium matrix and the internal membrane collagen. The damage is caused by strong pressing the metallic implant structure into the vessel wall and by additional friction forces between them. Adhesion and activation of thrombocytes, bonding of fibrinogen and, finally, a thrombus formation in the implant area occur as a consequence of the response to breaking the endothelium continuity and uncovering the connecting tissue fibres.

The recovery reaction after the vessel damage during the angioplasty leads to the creation of extracellular matrix and the formation of neointime. The latter is attributed to migration of smooth muscle cells and fibroblasts to the internal membrane, activated during the vessel damage. The analysis of the histological preparations revealed an irregular pattern of fibres in the neighbourhood of the hole made due to removing the stent. The nature of the lesions is an evidence of pressing the metallic implant structure into the vessel wall, which probably happens at the stage of the heart muscle systole that results in the outside pressure on the coronary arteries.

Heavy damage to the coronary vessel by the stent can induce the inflammatory process. In the histological preparations examined, in deeper layers of the coronary vessel, neutrocytes were observed that can participate in the inflammatory condition. The fully developed inflammatory condition itself was not observed. Most probably, infiltration of these cells was caused by the damage to the intracellular matrix elements, which enabled the migration of neutrophils.

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