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Research Article

Phenomena in Frozen Valves from A Valve Bank

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Abstract

Studies were conducted on valves from a valve bank, frozen for up to 19 years. Obtained results were compared to studies of fresh, non-frozen valves. Stereomicroscopy, polarized light microscopy, scanning microscopy and X-ray diffractometry were used in the studies. It was determined that the structures of valve leaflets after prolonged freezing time (4-5 years) do not return to their initial, pre-frozen state. Changes in the structures after freezing time of over 5 years may, when the valves are implemented, favor faster damage processes, including calcification. Hence it appears beneficial to only implant valves that have been frozen for less than 5 years.

Keywords: Heart Valves; Freezing

Introduction

Phenomena occurring in the heart, including its valves, may be connected to their biomineralization, commonly known as "calcification" [1-4]. Calcification is recognized using many methods [5-13]. Early and comprehensive diagnosis of changes in the heart is important; particularly essential is the choice of methods of recognizing calcification in specific parts of the heart – especially the valves and coronary vessels [14-21]. Recognition of the level of development of that phenomenon and the influence of the factors causing the calcification is the basis for choosing the right treatment method [20,22-25]. Particularly interesting are problems of valve mineralization, which causes a series of disorders not only of the heart but the whole circulatory system [26-30]. That is why, in addition to the pharmacological treatment [31-33], the choice of the right surgery technique and the right valve prosthesis [13, 34-40] is essential. One of the options is a frozen valve from a heart valve bank. This publication discusses some aspects of freezing heart valves and changes in the structures of frozen valves [41].

Materials and Methods

The material for research was gifted by prof. Dr hab. Roman Pfitzner from the John Paul II Hospital in Krakow, whom the author would like to offer sincere thanks. The studies were conducted on valve leaflets after the following periods of freezing altogether 19 valves:

a) Valves frozen in 2002 (15 years). Samples number 1873–1925.

b) Valves frozen in 2003 (13–14 years). Samples number 1928–1948.

c) A valve frozen in 2005 (12 years). Sample number 2031.

d) Valves frozen in 2009–2011 (9–10 years). Samples number 2149 – 2177.

e) Samples frozen in 2014–2015 (4–5 years, according to standards). Samples number 2232–2249.

f) Fresh valves, not frozen. Samples number 2290 and 2292.

The author's gratitude goes also to Mr. Adam Gaweł, MSci, for conducting X-ray analyses and results descriptions.

a) Digital Microscope

Preparations made from valves were examined using the Motic microscope of Chinese production, model 07-100477, with side light. Observations were made at various magnifications. Identified objects and phenomena were documented with photomicrographs using a Canon camera with a 20 MP matrix.

b) Scanning Microscope with EDS Detector

FEI Quanta 200 FEG microscope was used in the examinations.

They were conducted in the "Low Vacuum" mode, using cathodic voltage of 10 KV. Observed phenomena were documented with photomicrographs.

c) X-Ray Diffractometry

A Philips device with a vertical goniometer was used. Cu Kα radiation was used. Calculations of the dhkl values for individual reflexes were done based on X-ray data tables.

Results of Microscopic Studies

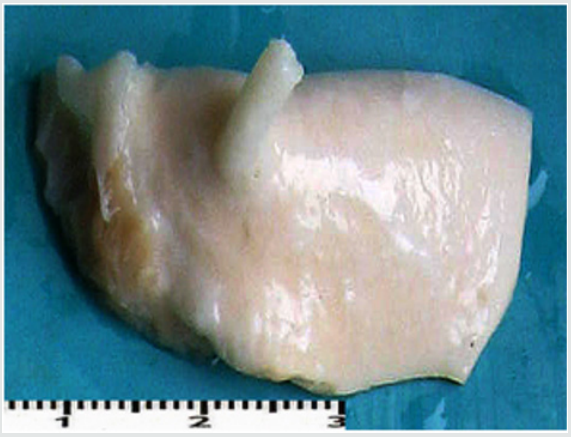

Photo 1(a): Sample photos of studied valves. A – fragment of a valve (sample 1925) after 15 years in deep freeze.

Macroscopic observations of the valve leaflets morphology showed that valves kept in deep freeze even for a long time do not differ from fresh valves (Photo 1). That suggested their full suitability for implementation. However, observations of the leaflets' surface conducted in higher magnifications reveal a lot of details, including differences in the appearance of the valve leaflets' surface. Apart from the visible differences resulting from the leaflets' structure, especially the layout of the collagen fibers (Photo 2(a)), defects connected with the process of harvesting the material for the valve bank are visible on the surface of some leaflets. The damaged spots are potential centers for calcification development (Photo 2(b)). Some of the defects are visible only in high magnification. With long-time freezing, they may favor deformation of collagen strands, which in result may shorten the time of correct functioning of the valve after being implemented. Observations of the valve leaflets under scanning microscope show that leaflets of the valves that have been frozen for a long time have, after defreezing, deformed structure of collagen fibers (Photo 3(a) & 3(b)). The degree of deformation depends on the freezing time (Photo 3(c) & 3(d)). Valves kept in deep freeze for a period of up to 5 years are almost unchanged or minimally changed structurally. Conducted observations also show that structural changes of the valve leaflets connected with long period of freezing affect not just the surface but also the fibrosa of the valves. It is visible in cross-sections of the leaflets (Photo 4(a) & 4(b)). Leaflets of valves that have been frozen for a long-time show, after thawing and in cross-section, separation of collagen fibers and often even their breaking. That means deterioration of physical parameters of the leaflets, which may result in worse efficiency of the valve made of that material.

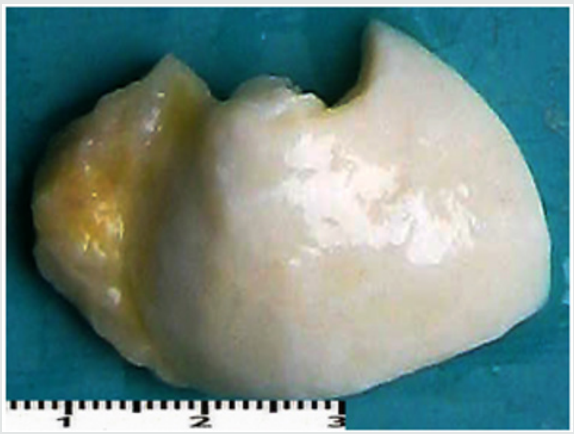

Photo 1(b): fragment of a fresh (not frozen) valve (comparison material).

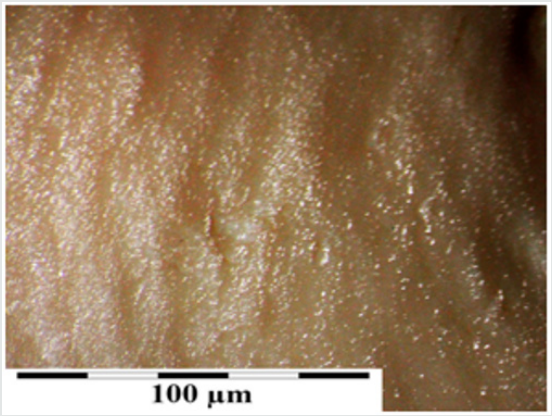

Photo 2(a): Sample photos of the surface of valve leaflets. Sample of the leaflet of a valve frozen for 15 years (sample 1925) with visible bundles of collagen fibers.

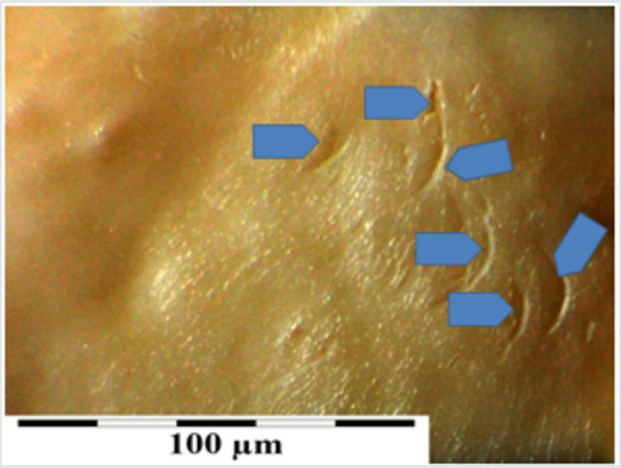

Photo 2(b): Surface of a valve leaflet (sample 2990) from a fresh, not frozen valve (comparison material). Arrows show places of damage from the process of valve harvesting.

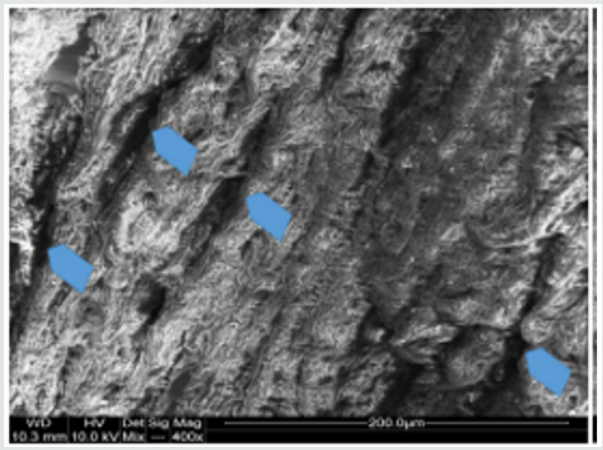

Photo 3(a): Sample photos of the surface of examined valves. Valve frozen for 15 years.

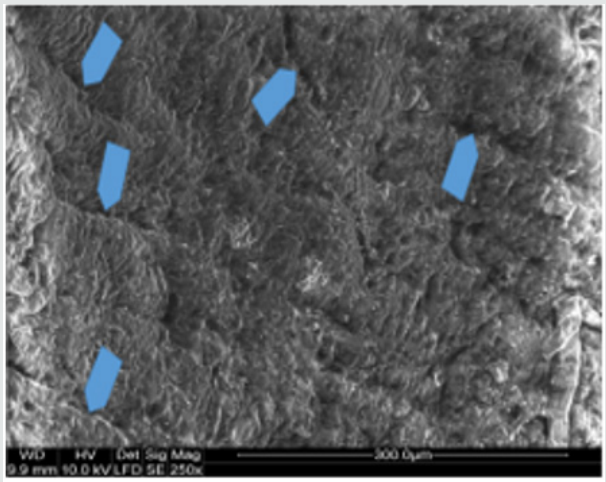

Photo 3(d): Valve frozen for 10 years.

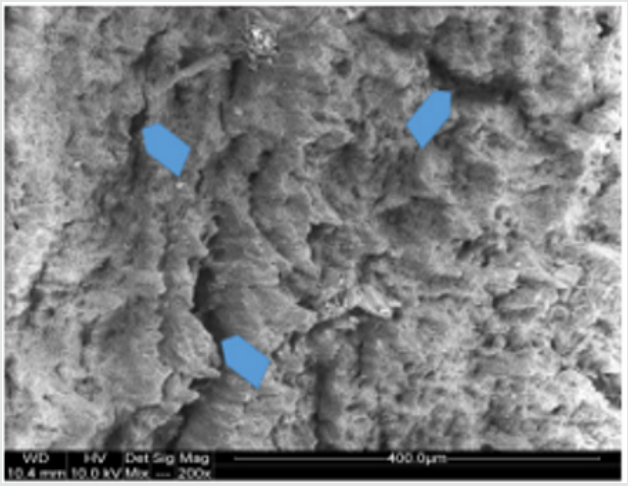

Photo 3(b): Valve frozen for 13 years.

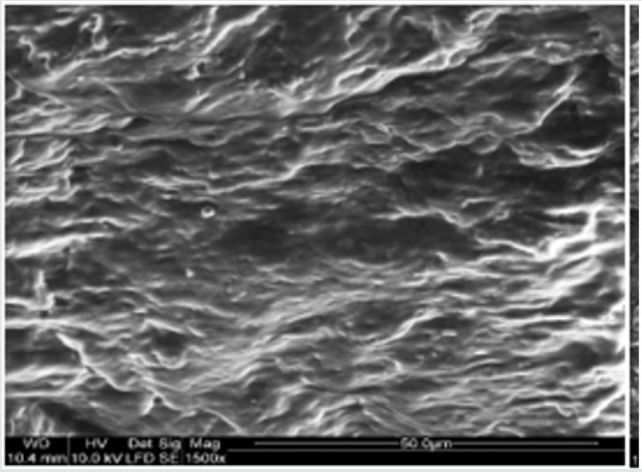

Photo 3(e): Fresh valves – comparison material. SEM.

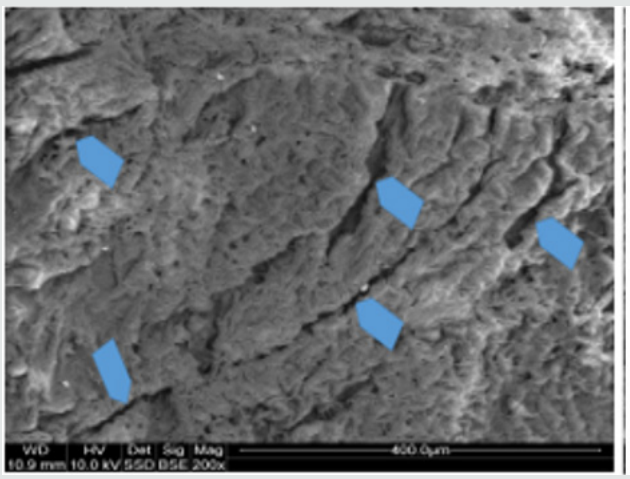

Photo 3(c): Valve frozen for 12 years.

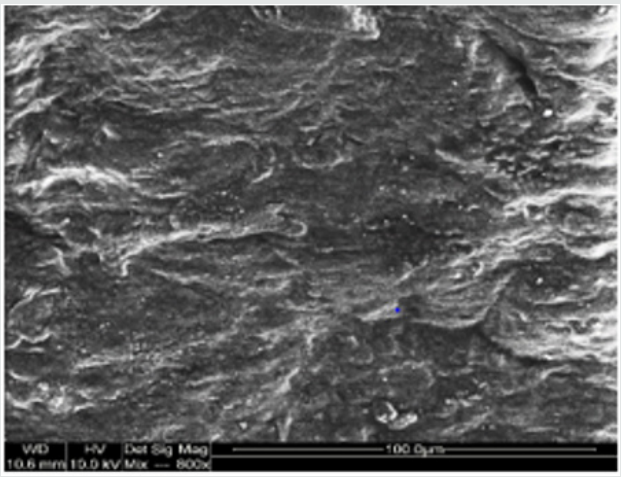

Photo 3(f): F – fresh valves – comparison material. SEM.

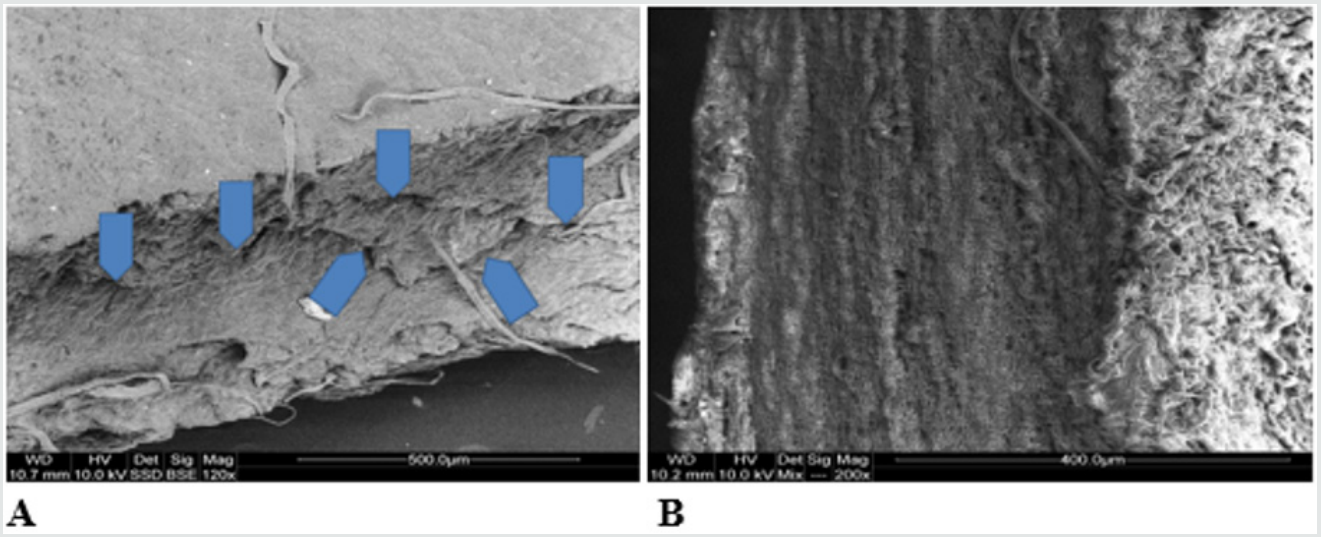

Photo 4: Sample photos of chosen valves in cross-section. A – cross section of a valve frozen for 12 years. Visible defects in valve fibrosa (arrows). B – fresh valve – comparison material. SEM.

Results of Structural X-Ray Diffractometry

The goal of X-ray examination was to recognize phenomena in the valve collagen depending on the period of freezing. From each of the valves (from a leaflet) a 1 cm by 1 cm square was cut and placed on the diffractometer head. The measuring was done within 0-75o 2Θ, obtaining a series of diffractograms (Figure 1). Despite seemingly identical character of the diffractograms, shifting of the diffused strand coming from water was observed, from 27o 2Θ to 30o 2Θ with increasing time of valve freezing (Figure 1). Described phenomenon indicates changing way of bonding water by the collagen structures of the valves with increasing freezing time. That phenomenon may be the result of deformation of collagen structure with prolonged time of valve freezing. On X-ray diffractograms of water present in the valves (Figure 1), it manifests with shifting of the maximum of the stronger diffused reflection from dhkl around 3,32Å to dhkl around 2.70 Å with the increasing time of valve freezing. Changes in this value may indicate shortening of interatomic bonds in collagen. Simply speaking, they indicate change of the structure of valve collagen after a prolonged time of valve freezing. That phenomenon in turn suggests that valves that have been frozen for a long time may be less durable in the recipient's body. Structural X-ray diffractometry of pure collagen conducted at the same time shows that (Figure 2) in addition to a strong peak of dhkl = 9.07 Å, which is also visible on the diffractograms of valves (Fig. 1), collagen gives a diffused band with heightened background within dhkl 7,37 Å - 3.08 Å. It hides under bigger and stronger band of water observed in valve diffractograms between dhkl 2.70 - 3,32 Å. Such situation impedes direct recognition by the X-ray method of structural changes in collagen connected with freezing. Hence the considerations regarding change of the structure of valve collagen connected with the freezing process are deductive in nature and are based only on observations related to structural changes in water bound by collagen of the frozen valves.

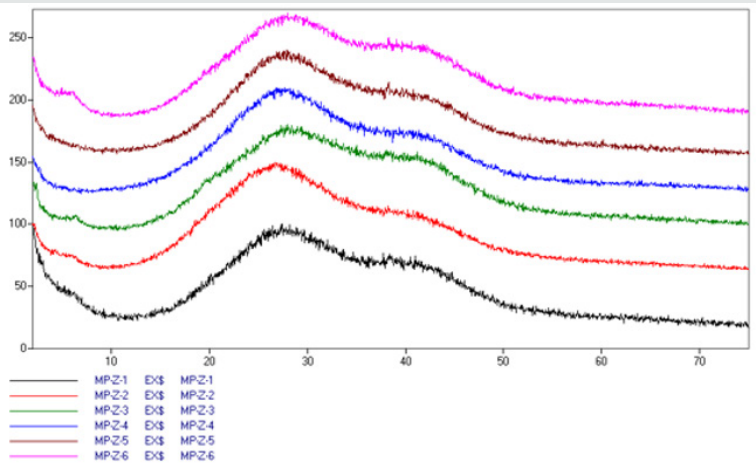

Figure 1: X-ray diffractograms of valve leaflets, presented from the bottom: Z – valve frozen for 15 years, Z5 – valve frozen for 5 years, Z6 – fresh valve. Visible oscillation of position and intensity of collagen peak (5o 2Θ) and change of position of maximum diffused water peak from 27o 2Θ to 30o 2Θ with the increasing time of valve freezing.

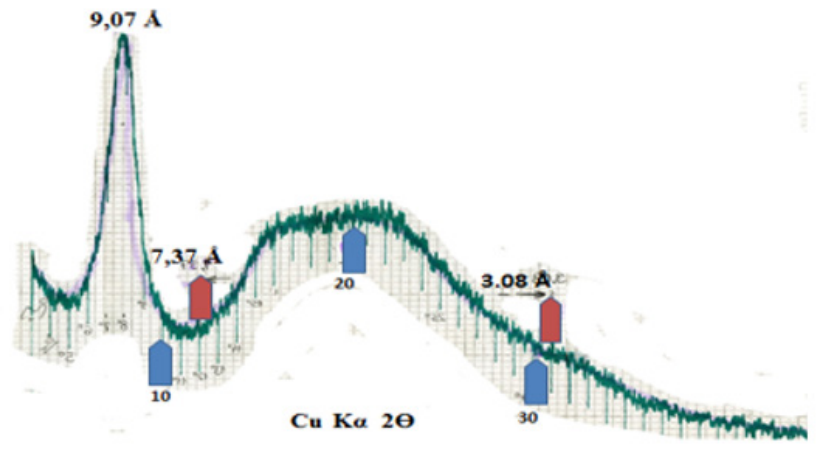

Figure 2: X-ray diffractogram of pure collagen.

Conclusion

SEM research indicates that deformation of collagen fibers in the valves takes place along with prolonged freezing time. Collagen fibers (in valves) frozen for a long time at a temperature of liquid nitrogen do not return to their original size after thawing. This affects the fiber length reduction to a smaller extent than their width. It results in the formation of micro-spacing between fibers, which after implantation, as a place with electric charges, may undergo calcification processes (biomineralization). Structural studies carried out using the X-ray diffractometry method indicate that long-term freezing promotes deformation of the structure of the collagen building the valve fibers, in addition to the deformation of the structure of collagen fibers. This is observed on X-ray diffractograms as a modified way of water binding on the surface of the valve leaflets kept for a long time in the frozen state. The obtained results can be "translated" into practical suggestions for transplants of allogeneic, frozen valves. They indicate that it is unfavorable to store the valves in freezing for a period longer than 4-5 years. Longer freezing of the valves causes changes that favor disadvantageous functioning of transplants.

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