Introduction

Plenty has been written about the impact of arteriosclerosis, i.e. arterial biomineralization, on the functioning of human body. A little less information is available concerning the dissolution and prevention of such mineralization (calcium channel blockers etc.) [1,2]. The significance of even a small progress in "cleaning" the arteries cannot be overstated. By renewing the proper functioning of the cardiovascular system, it will restore proper functioning of tissue, organs, and other elements of the body, and in effect support removal or reduction of many diseases. It will lead to lasting rejuvenation of the body.

Types of Arterial Biomineralization

Two types of mineralization present in arteries are mineralization with organic compounds (Figure 1) and/or mineralization with inorganic phosphorus compounds (Figure 1). However, the most common is organic-inorganic mixed mineralization with varying proportions of both types [3]. That hinders dissolution of deposits (arteriosclerotic plaque), because organic solvents are needed to dissolve organic deposits, while inorganic deposits require inorganic solvents. In the most common case of mixed arteriosclerotic plaque, combinations of organic and inorganic solvents need to be used [4-8].

Figure 1: A – concentration of cholesterol in arterial wall (arrows), B – cholesterol crystal aggregates formed on the surface of the intima, C – apatite crystal aggregates on the surface of the intima (arrows indicate places where chemical analyzes were carried out using EDS), D – phosphate aggregates on the intima surface.
Mineralization develops in biomineralization centers, which are places where different elements of artery structure have been damaged (39). Arteriosclerotic plaque is built from components transported with blood — mostly cholesterol, free Ca\(^{2+}\) and PO\(_4\)\(^{3-}\) ions, etc. It is interesting to note that biomineralization of both the intima and the arterial wall is not related to exceeding the solubility product of crystallizing components [9], because concentrations of those components in blood (mainly in plasma) are low. Since the formation of deposits occurs even at low concentrations of those substances in blood, it means that the mechanism that governs the biomineralization of arteries is slightly different than the one occurring after exceeding the so-called solubility product of crystallizing substance [10], where the concentration of components is so high that they must precipitate out of the transporting medium.

This situation causes an additional problem with the dissolution of deposits formed in the arteries. It is impossible to shift the system of chemical equilibrium in the blood in such a way that the number of components we want to dissolve from previously crystallized biomineralization is very low. Although that would undoubtedly promote the dissolution of deposits [11-16], it may disrupt the functioning of other organs in which circulating blood must contain a certain amount of the listed ingredients [17,18]. Considering the above, it was decided to first attempt to dissolve the deposits \textit{in vitro}, and then, in the future, use the obtained test results in attempts to dissolve arterial biomineralization \textit{in vivo} (Figure 2).

**Figure 2:** EDS energy spectrum of phosphate from the intima surface shown in Photo 1 D.

**Experimental Dissolution – Equipment and Method**

The apparatus shown in Figure 2 was used for experimental dissolution of arterial biomineralization \textit{in vitro}. It consists of a container with solvent (1), a peristaltic pump (2), a fragment of artery with cholesterol biomineralization or a tube with synthetic calcification (3), and a reservoir for solvent after its passage through an artery or tube with synthetic apatite (4). The solvent flow rate was adjusted to the average blood flow rate in human body [19-25]. The experiment was carried out at a temperature of 22 °C, in normal atmospheric pressure.

**Figure 3:** Drawing of a set of devices used in the experimental dissolution of arterial biomineralization. 1 - container with solvent, 2 - peristaltic pump, 3 - fragment of artery with cholesterol biomineralization or tube with synthetic calcifications, 4 - container for solvent after it flows through the system (4). Arrows show the direction of solvent flow.
The experiment included both mineralization occurring in arteries and synthetic apatite mineralization placed in a tube (Figure 3). Testing was carried out before and after passing the solvent through. Tests were also carried out on the products of crystallization from the solvent after it had passed through the biomineralization zone [25-31]. Polarization microscope (Meiji microscope manufactured in China) and scanning microscope (Jeol 540 and 560 microscope of Japanese production) were used in the research [32-36].

Results of the experiment are presented as follows:

a) Dissolution of organic biomineralization

b) Dissolution of phosphate (apatite) mineralization

Dissolution of organic biomineralization

Dissolution of organic deposits occurring on walls of arteries used in experiments was preceded by tests regarding presence of cholesterol deposits [36-41]. Many different solvents were passed through the arteries (Figure 4), but only the results of the best dissolution effects were presented in this publication. Fragments of arteries were obtained due to the kindness of cardiac surgeon prof. Roman Pfitzner from the John Paul II Hospital in Krakow, for which the author gives heartfelt thanks [42]. Ethyl alcohol proved to be one of the best cholesterol and fats solvents. After pumping through the arteries, it was evaporated, and the resulting microcrystals were examined. The studies showed that the crystals were represented by microcrystalline cholesterol (Figure 5).

Figure 4: Fragment of an artery affected by significant cholesterol biomineralization, used in the experiments (arrows).

Figure 5: Cholesterol microcrystal crystallized out of the ethanol that was pumped through arteries affected by cholesterol biomineralization.

Dissolution of phosphate (apatite) mineralization

Experiments with dissolving phosphate biomineralization proved to be complicated, because in the studied arteries such mineralization never occurred without accompanying cholesterol. In order not to complicate the experiment [43-49], it was decided that apatite would be synthesized, and an “artificial” artery covered
by phosphate mineralization would be created. Arterial phosphate biomineralization [50], which in previous studies proved to be common (although usually co-occurring with organic compounds), turned out to be very complicated to synthesize outside the body. Many experiments allowed us to obtain a phosphate gel, which during crystallization led to the synthesis of microcrystalline carbonate apatite where about 10% of $\text{PO}_4^{3-}$ groups were replaced by $\text{CO}_3^{2-}$ groups (Figure 6). Size of the crystals of this apatite was regulated through the time of crystallization [51,52], which was carried out at the temperature of 30 $^\circ$C. Mineralogical studies confirmed total mineralogical compatibility of the obtained synthetics with apatite formed in the arteries.

Grains of the synthetic apatite were placed inside a plastic tube, which was then attached to the experimental dissolution system (Figure 7 & 8). Experimental dissolving of synthetic apatite was attempted with many solvents. The best one proved to be distilled water [53-56]. The surface of apatite grains removed from the tube was studied after their experimental dissolution with distilled water as a solvent. Observations conducted with scanning microscopy confirmed dissolution of apatite grains. It manifested on their surface as a series of morphological depressions, channels and irregularities (Figure 9).
Summary

Presented research results are an experimental contribution to better understanding of the phenomena of crystallization and dissolution of arterial biomineralization, which is one of the main research problems concerning the circulatory system. The experiments that were carried out indicate the possibility of dissolving both cholesterol and apatite deposits found in human arteries. Depending on the type of arterial biomineralization, different "solvents" should be used: organic solvents for organic substances, inorganic for apatite deposits [57]. It is probably also possible to use mixed composition solvents that will dissolve the most common type of arterial biomineralization, i.e. organic-inorganic mixed mineralization. However, that requires further experimental research. Experimental synthesis of apatite needed for presented research allowed us to determine the conditions of both its crystallization and dissolution (Figure 10). They explain why apatite deposits do not form in veins [58]. Venous blood contains CO₂ and other products of metabolism that cause its slight acidification. Lower pH is enough to prevent phosphates, including apatite, crystallizing in the veins. The range of apatite instability is pH 6.6-6.8.
Since it is impossible to lower the arterial blood pH to such values, other elements should be considered when thinking about dissolution of arterial biomineralization [59-61]. In case of calcium phosphates, the body should be stimulated in such a way that the level of ionized calcium (Ca\(^{2+}\)) in the blood plasma is reduced (without detriment to the body's functioning). It appears that thyroid and the parathyroid glands may play an important role here. Situation with cholesterol as a derivative of bile acids synthesized by the liver is more difficult. Shifting the chemical balance of arterial blood towards lowering the level of cholesterol does not eliminate the cause, which is dysfunction of the liver that causes intense cholesterol synthesis. It seems important to stimulate the liver in such a way as to reduce the amount of bile acid in the bile, which is responsible for the synthesis of cholesterol and its transport in the bloodstream. The problem is complex and requires further multidirectional research.

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Figure 10: Diagram of the pH balance of the solution - experimental crystallization and dissolution of calcium apatite with some of PO\(^4^-\) groups replaced by CO\(^3^-\) as well as F\(^-\), Cl\(^-\) and OH\(^-\) ions.

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