

Review

Selenium-Doped Hydroxyapatite Nanocrystals—Synthesis, Physicochemical Properties and Biological Significance

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Abstract: Hydroxyapatites (HAs), as materials with a similar structure to bone minerals, play a key role in biomaterials engineering. They have been applied as bone substitute materials and as coatings for metallic implants, which facilitates their osseointegration. One of the beneficial characteristics of HA, when used to create biocompatible materials with improved physicochemical or biological properties, is its capacity for ionic substitution. The aim of the study was to present the current state of knowledge about HAs containing selenate ions IV or VI. The enrichment of HAs with selenium aims to create a material with advantageous effects on bone tissue metabolism, as well as having anticancer and antibacterial activity. The work is devoted to both methods of obtaining Se-HA and an evaluation of its chemical structure and physicochemical properties. In addition, the biological activity of such materials in vitro and in vivo is discussed.

Keywords: hydroxyapatite; ionic substitution; selenium; selenite; selenate; physicochemical properties; biomaterials; bioceramics

1. Introduction

Calcium hydroxyapatite (HA), with the general formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, belongs to the group of crystalline calcium phosphates [1]. Until recently, it was considered to be the main inorganic component of bone tissue and mineralized dental tissues. However, it turned out that biological apatite is characterized by a more complex composition; nevertheless, synthetic HA has been used as a bone replacement in orthopaedics, implantology, regenerative medicine, and dental surgery [2].

Hydroxyapatite is characterized by its high biocompatibility with bone tissue and its complete non-toxicity. Importantly, it has osteoconductive properties, stimulating bone tissue to grow. Therefore, HA is one of the basic materials used to cover metallic periosteal implants [2,3]. It is also used to create bone substitute materials and implants; however, its fragility limits its application only to areas with low mechanical stresses. In turn, one of the beneficial features of HA-based materials is their porosity, which depends, inter alia, on the method of synthesis and preparation. This feature is used in the development of bone drug delivery systems [4].

This work focuses on synthetic HA modified with selenium ions IV and VI. Our aim was to present the current state of knowledge on selenium's effect on the structure of HA and on its biological properties.

1.1. Hydroxyapatite—Structure and Function

Pure, stoichiometric HA crystallizes in a monoclinic system, space group $\text{P2}_1/\text{b}$ [5]. However, it is worth mentioning that HA like this is rare and very difficult to obtain by synthesis.

The most common type of HA is the type that crystallizes in the hexagonal system, space group $P6_3/m$ [5]. The crystallographic structure is well known and has been described in many articles [6,7]. In short, hydroxyl ions are located in the corners of the rhomboidal cell base (Figure 1). Six calcium ions are associated with hydroxyl groups from the unit cell corners. These calcium atoms (denoted in the literature as $\text{Ca}(2)$) form equilateral triangles situated perpendicularly to the “ c ” axis, and moved away from each other by 60° . The remaining four calcium atoms are arranged in two separate columns along the “ c ” axis. They are surrounded by six oxygen atoms derived from phosphate ions. Phosphorus atoms surrounded by four oxygen atoms occupy most of the space between the calcium ions, creating almost regular tetrahedrons. The parameters of the crystal lattice are as follows: $a = b = 9.432$, $c = 6.881$ Å, $\gamma = 120^\circ$ [5,6].

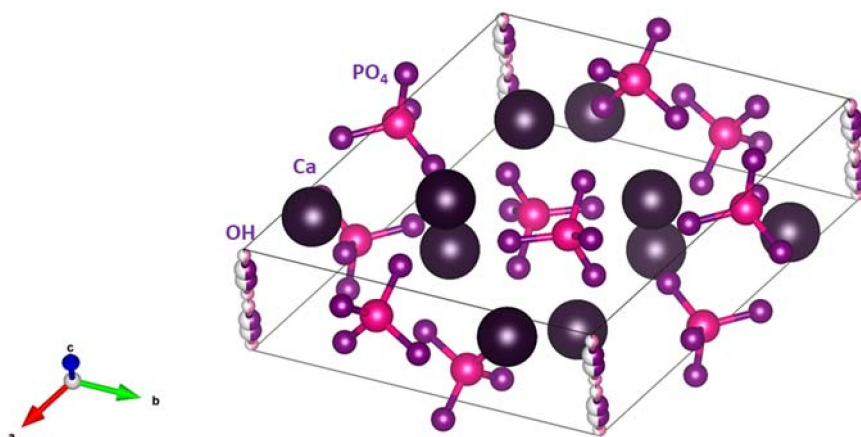


Figure 1. Crystallographic structure of HA.

The characteristic feature of HA is its susceptibility to ion substitutions, both in place of anions and cations, in the crystal lattice [7]. Certainly, ion exchange affects the size of the unit cell, the lattice parameters and, thus, the size of the crystallites. It also affects the physicochemical properties of HA, such as its solubility, thermal stability, surface area. Additionally, it is very important that “foreign ions” introduced into the HA structure can provide new biological properties. For example, silicate ions (SiO_4^{4-}) stimulate the proliferation of osteoblasts, thus improving the bioactivity of HA [8]. Silver ions have a strong bactericidal effect on most Gram(−) and Gram(+) strains, which can be used in the production of biomaterials with additional antibacterial properties [9].

1.2. The Role of Selenium in Human Organisms

Selenium is one of the essential microelements determining the proper functioning of the human body. The characteristic feature of selenium is its narrow therapeutic window—there is a very small difference between a concentration having a beneficial effect on the human body and one at which toxic activity is exhibited [10]. Selenium exhibits antioxidant properties and protects the body against free radicals and carcinogens. It contributes towards many biochemical processes as an important component of the 25 known selenoenzymes, e.g., glutathione peroxidase or iodothyronine deiodinase [11–13]. To date, there have been reports on the positive effects of selenium on cardiovascular diseases, cancers, the thyroid, the brain, reproduction, bone tissue, and viral infections, as well as on the immune system (Figure 2).

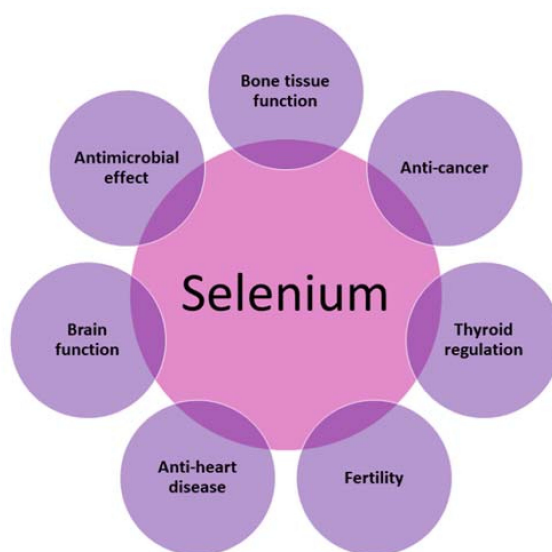


Figure 2. The health benefits of selenium.

Selenium also has immunostimulatory properties. It has been demonstrated that it increases the proliferation of activated T lymphocytes, increases the cytotoxicity of cancer cells, and also activates NK cells. The possible mechanism of the immunostimulatory action of selenium involves an increase in the expression of interleukin 2 receptors, which induces the proliferation of T lymphocytes [14,15]. It was proven in studies on mice that those on a diet rich in selenium showed an increased expression of interleukin 2, which was accompanied by a strengthened signal from T lymphocytes [16].

It was proven that selenium deficiency affects the development of epileptic seizures, and Parkinson's disease, as well as leading to coordination problems and a decline in cognitive skills [17,18]. Selenium also has a significant effect on male fertility [19,20]. It participates in the biosynthesis of testosterone, as well as in the formation and development of spermatozoa [19].

The thyroid is the organ containing the highest concentration of selenium. As mentioned, selenium is a component of selenoenzymes—for example, deiodinase, which is involved in the production of active thyroid hormones—and triiodothyronine from an inactive precursor, thyroxine. Moreover, selenium in the form of glutathione peroxidase protects the thyroid cells against hydrogen peroxide, which is formed as a result of the synthesis of thyroid hormones. An excess of this compound could cause the destruction and fibrosis of the gland [13,21,22].

Studies have indicated that low blood levels of selenium are also associated with myocardial infarction incidents and an increased risk of death due to cardiovascular diseases. Selenoproteins prevent the oxidative modification of lipids, inhibit platelet aggregation, and inhibit inflammation [21,23]. Therefore, selenium, through glutathione peroxidase, helps to protect the endothelial cells of blood vessels from the deposition of oxidized low-density lipoproteins arising from the oxidation of phospholipids and cholesterol esters. Therefore, it prevents atherosclerosis and its consequences [24–26].

The development of cancers is mediated by, inter alia, oxidative stress, as well as impaired bodily protective functions [13,27]. In recent years, scientists have been conducting intensive research on the effect of selenium on the reduction of colon, lung, liver, thyroid, and prostate cancer. Czczot et al. [27] found a decrease in the activity of glutathione peroxidase in hepatic liver tissue when compared to healthy tissue. This weaker level of enzyme activity may result in enhanced lipid peroxidation and an increase in the number of final peroxidation products, such as malonic aldehyde (MDA). Increased MDA levels were observed in tumour tissue. The studies prove that there may be many mechanisms by which selenium inhibits carcinogenesis and improves the effectiveness of

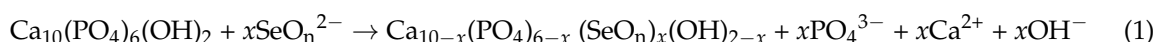
cytostatic drugs, as well as reasons why the relationship between selenium dose and tumour growth is not linear [24,28].

Selenium is also essential for the proper functioning of bone tissue. It has been proven that selenium deficiencies may delay growth and affect the metabolism of bone tissue [29]. The mechanism of these processes is associated with the function of selenoproteins, of which at least nine are expressed in human foetal osteoblasts. Their expression seems to protect bones from oxidative stress, which is important in the regulation of inflammation and the differentiation of bone cells. An excessive level of intracellular reactive oxygen species (ROS) may contribute to the development of osteoporosis by inhibiting the differentiation of osteoblasts in bone marrow stromal cells [30,31]. It was found that the concentration of selenium in plasma is inversely proportional to the rate of bone tissue turnover and positively correlated with the incidence of low bone mineral density in healthy postmenopausal women [32]. Selenium may play an important role in cells, especially at doses higher than those required for the maximal expression of selenoproteins. It can induce cell-cycle inhibition, apoptosis, immune function or the prevention of bone resorption through the inactivation of osteoclasts. These processes can provide potential protection against rheumatoid arthritis, osteoarthritis, or osteoporosis [31].

2. Synthesis of Hydroxyapatites Doped with Selenite and Selenate Ions

HA doped with selenium ions can be obtained in two ways: through synthesis, during which ions are incorporated into the HA structure [33–47], or by ion exchange, where soaking in a selenium salt solution results in the exchange of ions in the HA structure [47].

Theoretically, there are two positions in the unit cell of the HA crystal in which it is possible to incorporate selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) ions: into the structural channels of OH groups along the *c*-axis or in the orthophosphate site [29]. SeO_4^{2-} ions have a structure geometrically similar to the PO_4^{3-} ion, although they are much larger and measure 249 pm in diameter compared to the diameter of 238 pm of the PO_4^{3-} ion [42,43,48]. SeO_3^{2-} ions have a similar diameter to that of PO_4^{3-} ions, measuring 239 pm; however, they differ in their geometric arrangement and have a flat trigonal pyramid structure [43,48,49] (see Figure 3). Looking at the size of SeO_3^{2-} and SeO_4^{2-} ions, their incorporation into the structure of HA is possible, but, as shown in the model diagram by Kolmas et al., only in place of phosphate ions [40]. Moreover, the authors noticed that, in the case of PO_4^{3-} ion substitution with SeO_3^{2-} or SeO_4^{2-} ions, we substitute a triple charge ion with a double charge ion which, in turn, leads to the simultaneous removal of Ca^{2+} and OH^- ions according to Equation (1) [43]:



where *x* is the content of selenium oxyanions, and *n* may be 3 or 4 depending on whether a selenite or selenate ion has been substituted.

Studies on the adsorption of selenium ions to the surface of HA crystals have examined both the adsorption of selenite and selenate ions [50–52]. These studies have confirmed that these ions are incorporated in the place of phosphate groups, but it was surprising that SeO_3^{2-} or SeO_4^{2-} ions do not stay on the surface, but diffuse into the interior to a depth of a few nanometres [51]. Moreover, the sorption capacity of the selenite ions was significantly higher than that of selenate ions [52].

During Se-HA synthesis, selenium-doped HA was most frequently obtained (both in the form of SeO_3^{2-} and SeO_4^{2-}) using the wet method with a co-precipitation reaction.

The obtained powders contained various amounts of selenium [33–47], most often up to 10% by weight [33,36,37,40,41,43,44,46]. It should be noted that the reaction efficiency was often lower than 100% [33,36,40,41,43,44,46]. Ma et al. obtained a SeO_3 -HA series starting from a small concentration of 3% of Se-substituted phosphate ions up to the almost full replacement of P ions with Se ions using a molar ratio of Se:P at 100:1 during the synthesis [45].

It is worth noting that the $\text{SeO}_3\text{-HA}$ obtained in the studies [34,38,39] was used to prepare biocomposites containing silk fibres [34] or lysozyme [39]. It was possible to incorporate between 6 and 30% of selenium ions relative to phosphorus ions into such materials.

Zhang et al. [42] slightly modified the wet method. The precipitated crystals were treated hydrothermally for 36 h at 160 °C. By so doing, selenium ions were incorporated in a molar ratio of Se:P ranging from 0.001 to 0.421 [42]. The solvothermal method of heating for 10 h at 120 °C was also applied by Sun et al. [35]. The reaction was carried out in a Teflon-lined reactor with the addition of PEG 20000 and oleic acid in substrate proportions that allowed a series of samples to be obtained that contained between 0% and 55% of phosphate ions replaced with selenite ions [35].

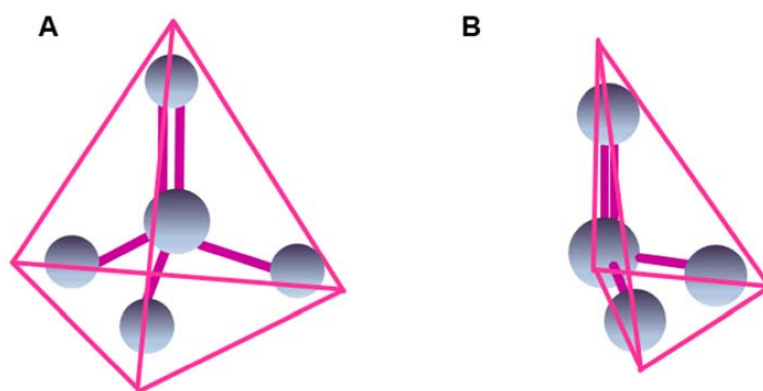


Figure 3. Tetrahedral (A) and trigonal pyramid (B) arrangements of phosphate and selenite (A) and selenite (B) ions.

In the studies [33,37,40], a dispersion agent was used in the wet method in the form of either sodium polyacrylate [37,40] or PEG [33]. In turn, the post-precipitation method, in which the surface phosphate groups are exchanged with selenium ions, was used by Uskokovic et al. [47]. They immersed previously precipitated HA crystals in a sodium selenite solution (pH ~10) while maintaining the solid phase content within the range of 10–40% *w/w* for a period of 48 h. In this way, a maximum of 0.36% by weight of selenium was incorporated into the hydroxyapatite structure [47].

In the case of surface HA coating, the PLD (pulsed laser deposition) application of powders, consisting of a mixture of HA and selenium powder, is used [53,54]. In such coatings made on titanium or silicon, the plan was to incorporate up to 2.7% of selenium [53,54]. The apatite material was applied using the PLD method under reduced pressure in a steam atmosphere, heating the carrier up to 460 °C using a laser with a wavelength of 193 nm [53,54].

HA coatings can also be obtained by soaking carried out in a simulated body fluid (SBF) doped with selenate ions [55].

3. Physicochemical Examination of Hydroxyapatites Doped with Selenate and Selenite Ions

In order to confirm the HA structure during the incorporation of selenium ions, the samples were analysed using powder diffractometry (PXRD). Based on the resulting diffractograms, the size of the obtained crystallites, the crystallinity, as well as the size of the unit cell of the obtained crystals, were also calculated. The morphology of the obtained crystals can be described using images taken via transmission and scanning electron microscopy (TEM and SEM). In order to confirm the incorporation of SeO_3^{2-} and SeO_4^{2-} ions into the HA structure, spectra were performed by using medium-infrared spectroscopy, Raman spectroscopy, and nuclear magnetic resonance imaging. Elemental analysis of the samples was performed using energy-dispersive X-ray spectroscopy (EDS), X-ray fluorescence spectroscopy (XRF) and X-ray photoelectron spectroscopy (XPS), as well as the titration method.

3.1. Powder Diffractometry (PXRD)

In each of the discussed studies, it was confirmed, based on a comparison of the obtained results with a reference sample containing no selenium ions and/or with a model HA diffractogram originating from ICDD and JCPDS databases, that monophasic material was obtained with a preserved $P6_3/m$ crystallographic system characteristic of HA.

In the study [43], diffractograms obtained for samples doped with selenate and selenite ions were compared with a diffractogram of standard HA and it was shown that the former have weakly separated wide reflections and that some of them are too wide to be distinguishable. Based on the Scherrer equation, the crystal dimensions were estimated using the reflexes (002) and (130/310) along the c and a axis, respectively. It was found that, after the introduction of selenite and selenate ions, the size of the crystals was similar to the size of crystals of biological apatites in bone tissue [43]. However, a decrease in the size of crystallites in comparison to pure HA was observed in the study [36], with dimensions of below 10 nm. The crystallinity of the obtained HA was highest for pure HA and lowest for SeO_3 -HA [41].

The powder diffraction method was also used in [34,37–39,44,46] to confirm the obtaining of monophasic HA material. The widening of reflections was observed as selenite concentration increased, which indicates a decrease in the size of crystallites, as well as a decrease in crystallinity [34,38]. The sizes of the SeO_3 -HA crystallites, calculated using the same method as in [46], are approximately 20 nm and are not much smaller than in pure, unsubstituted HA. A decrease in crystallite size along the c axis with an increase in Se concentration was also observed in the HA-lysozyme composites [39].

Ma et al. [45] attempted the almost total exchange of phosphate ions for selenite. Based on a comparison with the reference diffractogram, the behaviour of the hydroxyapatite monophase was determined to be highest at an Se:P ratio of below 10 [45]. At higher concentrations, reflections characteristic of calcium selenite were observed [45]. In the samples in which the HA structure was preserved, the lines were significantly widened.

Similar results were achieved by Wei et al. [33]. They observed the reflections from the HA phase on the diffractogram in samples with a molar Se:P ratio of a maximum of 0.3 (Figure 4). In samples with a Se:P value > 1 , reflections from calcium selenite were observed. The paper also describes samples that were heated at a temperature range from 700 to 1200 °C, which resulted in the sharpening of reflections, proving that the crystallinity of samples increased. In some samples heated at temperatures between 1100 and 1200 °C, the occurrence of the β -TCP phase was noticed [33].

Zhang et al. also confirmed the production of SeO_3 -HA in all samples using the PXRD method. The calculated crystallite size decreased from 45.3 to 17.4 nm [42]. As in other studies, it was noticed that the reflections became wider with increasing selenium concentrations. The results obtained with the PXRD method were additionally confirmed by the SAED electron diffraction method [42].

Samples of SeO_3 -HA obtained by the solvothermal method, with 15–40% of the phosphate ions being exchanged for selenite, have reflections typical of HA [35]. In the sample with 55% P exchanged for Se, additional reflections were noted from the calcium selenite [35].

Uskokovic et al. [47] confirmed the achievement of a monophasic hydroxyapatite material for samples with concentrations of SeO_3^{2-} ions ranging from 0.1% to 1.9%. However, the sample with SeO_3^{2-} concentration of 3% additionally contained reflections from the calcium pyrophosphate phase ($\beta\text{-Ca}_2\text{P}_2\text{O}_7$), which became visible only after calcination at 800 °C. The widening of reflections with increased SeO_3^{2-} ion content was also noticed [47]. The authors suggest that the decrease in crystallinity with an increase in the content of selenium ions is caused by an uneven dissolution rate in various environments. In an acidic environment, much more Ca^{2+} ions than PO_4^{3-} ones are released, while in an alkaline environment, this mechanism is reversed. However, during the formation of HA, the first stage is the creation of a phosphate-based lattice with incorporated calcium ions, and this process is promoted by a higher concentration of phosphate ions. In turn, the replacement of some of phosphate ions with selenium ions during synthesis reduces the concentration of PO_4^{3-} ions, which causes the disturbance of the structure growth and as a result, the macro structure

is disturbed (long-range order). In addition, this may hinder recrystallization, as a result of which the amorphous particles change into larger crystalline structures. The result of this is that the transformation and preservation of the amorphous structure is inhibited. The authors see another possible cause of structural disruption, i.e., only a few double-charged SeO_3^{2-} ions are incorporated into the crystal structure.

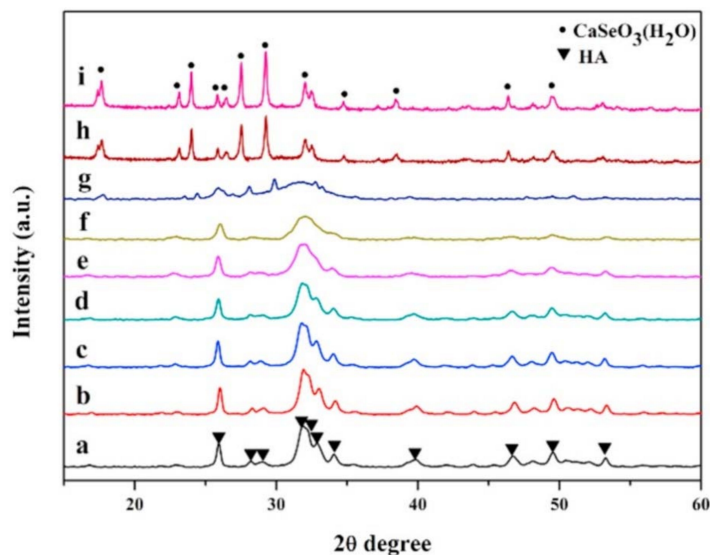


Figure 4. XRD patterns of the pure HA and Se-HA samples with different Se/P ratios: (a) HA; (b) Se/P = 0.05; (c) Se/P = 0.08; (d) Se/P = 0.1; (e) Se/P = 0.3; (f) Se/P = 0.5; (g) Se/P = 1; (h) Se/P = 3; and (i) Se/P = 5. Reprinted from [33] with permission from Elsevier.

Based on the above-described diffractograms, the crystallite size for samples obtained by both co-precipitated and post-precipitated methods [47]. In both types of samples, the incorporation of selenite ions causes a decrease in the size of crystallites, but this is not correlated with the content of these ions in the sample [47].

Coatings obtained by soaking in SBF were analysed using the PXRD method and they were then compared with the reference HA diffractogram [55]. The reflections for the obtained HA and SeO_4 -HA coatings are typical of amorphous HA; they have wide lines with a position characteristic of the HA phase. Since the width of the reflections on the diffractogram of the SeO_4 -HA coating does not differ from the reflections of the HA coating, the authors claim that both materials have a similar crystallinity.

3.2. Examinations Using Electron Microscopy Methods (SEM, TEM)

Electron microscopy is a frequently used method when analysing materials with a crystalline structure, since it allows for an evaluation of the morphology and size of the crystals. The materials obtained in [36,43] had plate-shaped nanometre crystals; their dimensions did not exceed 10 nm in width and 30 nm in length [43]. The crystals were characterized by a strong tendency to form dense agglomerates. In turn, Wang et al. obtained crystallites similar in shape to needles during the synthesis of SeO_3 -HA, through the linear aggregation of single crystallites [38]. The dimensions of such nanoparticles were determined, based on TEM images, to be 20–30 nm in width and 150–200 nm in length [38,44,46]. Examinations of the size of nanoparticles using the dynamic light scattering (DLS) method indicated that there was a minimal increase in particle size in samples containing selenium ions, that it caused by a high tendency to agglomerate [38,56].

In the study [37], the synthesis method was modified by adding a dispersant, which enabled the formation of needle-shaped crystals with dimensions of below 100 nm, as confirmed by DLS and TEM measurements.

TEM and SEM images of a composite based on SeO_3 -HA and silk fibres showed that in the samples without selenite ions, the crystals agglomerated as polycrystalline bamboo-shaped needle bundles [34], while the sample containing 6% selenium also contained needle-shaped crystals; however, they were more clustered and proteins derived from silk fibres covered the surface of the polycrystal like a thin film. For samples with higher concentrations of selenium, a related ribbon structure with the appearance of a large grid was observed [34]. Structural disorders were also noticed in the form of separated large crystals. Simultaneous SEM images confirmed that silk fibres covered the surface of polycrystalline HA, forming a porous grid structure [34].

In contrast, HA-lysozyme composites [39] obtained by Wang et al. had narrow, sharp needles which tended to agglomerate; the dimensions, measured based on TEM images, were 10–25 nm in width and 120–150 nm in length.

Photographs taken using the field emission scanning electron microscopy (FE-SEM) method show the very irregular surfaces of composites that can be formed during the drying process, when the water molecules evaporate from the loosely adhesive interface. Images taken with this method also indicate the agglomeration of nanocomplexes.

Needle crystals were obtained in the study [40]. It was also observed that an increase in selenium content increased the size of the crystals and intensified their agglomeration [40].

The shape of the 150–200 nm long needles was recorded in TEM images by Wei et al. [33]. The authors compared samples with the Se:P molar ratios of 0.3 and 0.5, and noticed that an amorphous fraction appeared on the surface of crystals in a sample with a higher selenium content, which was confirmed by results obtained using the PXRD method.

Zhang et al. obtained rod-like crystals using the hydrothermal method, and based on SEM and TEM images, their length and width were measured to be 50 and 12 nm, respectively [42].

However, the use of the solvothermal method resulted in rod-like nanoparticles with dimensions of 8 to 150 nm [35]. Low concentrations of selenium (up to 15%) exchanged with P caused the rods to narrow and the ends to tighten, while an increase in the content of incorporated selenium to 40% caused a change in shape, where the rods transformed into needles of up to 100 nm in length. The authors of the study [35] also verified that the reaction temperature had an influence on the morphology of the nanoparticles. After reducing the reaction temperature to 100 °C, the obtained nanoparticles were shaped like rods. On the other hand, gradually raising the temperature to 200 °C resulted in the particles maintaining their length but gradually becoming narrower.

Uskokovic et al. compared images of pure HA and SeO_3 -HA recorded using the TEM method, and noted that pure HA is composed of two types of crystals with particles of approximately 5 nm and elongated crystals with dimensions of 10–15 nm $\times$ 50–100 nm [47]. The coexistence of the two forms is indicative of aggregative crystal growth and is the result of the tendency to grow mainly along the *c* axis of the crystal. In contrast, SeO_3 -HA obtained using the wet method had homogeneous grains measuring 20–50 nm in diameter. This is probably due to the fact that SeO_3^{2-} ions block the aggregation of small particles into more crystalline needles, which causes the resulting SeO_3 -HA to be less crystalline than pure HA.

The HA and SeO_3 -HA coatings obtained by the PLD method on Ti and Si materials were analysed by SEM [54]. The researchers described the surface morphology as being typical of HA, consisting of spherically shaped aggregates; moreover, the similarity of the HA and SeO_3 -HA coatings indicated a similar mechanism of formation by globular groupings.

Coatings deposited using SBF doped with selenium ions were analysed based on field emission FE-SEM images [55]. The researchers noticed the first calcium phosphate compounds after four days. It turned out that, after 14 days of soaking, the whole surface was covered with semicircular compact SeO_4 -HA with a homogeneous morphology. When comparing the coatings, the authors noticed that their thickness increased with longer soaking times, and that the coating based on Se-doped SBF was more homogeneous [55].

3.3. Examinations Using Mid-Infrared Spectroscopy (FT-IR)

HA samples doped with selenium were analysed using mid-infrared spectroscopy in the range of 4000–400 cm^{-1} [36,38,43,45,46]. Based on the reference spectrum of hydroxyapatite, the occurrence of HA-specific bands was confirmed (see Table 1). Additionally, Kolmas et al. [36,43] recorded two bands at 767 cm^{-1} and 840 cm^{-1} on mid-infrared spectra for HA doped with SeO_3^{2-} ions, the intensity of which increased with the content of selenite ions (Figure 5). These bands were assigned to symmetrical and asymmetrical vibrations of the Se–O bond of selenite ions in HA crystals. In turn, a band at approximately 505 cm^{-1} was attributed to bending vibrations [43]. For the HA doped with selenate ions, an additional band at 910 cm^{-1} was recorded that was not observed on the pure HA spectrum, which was attributed to the Se–O stretching vibrations of the SeO_4^{2-} group. Based on the location of these bands, the authors confirmed that selenite and selenate ions had become incorporated into the structure of HA crystals.

Table 1. Main FT-IR bands of HA [36,38,43,45,46].

Bands (Wavenumber cm^{-1})	Assignment
3700–2500	ν_3 and ν_1 stretching modes of hydrogen-bonded H_2O molecules
3570	stretching modes of structural hydroxyl groups
1630–1640	Bending modes of hydrogen-bonded H_2O molecules
1200–900	ν_3 and ν_1 of PO_4^{3-}
605–500	ν_4 PO_4^{3-}
475–470	ν_2 PO_4^{3-}

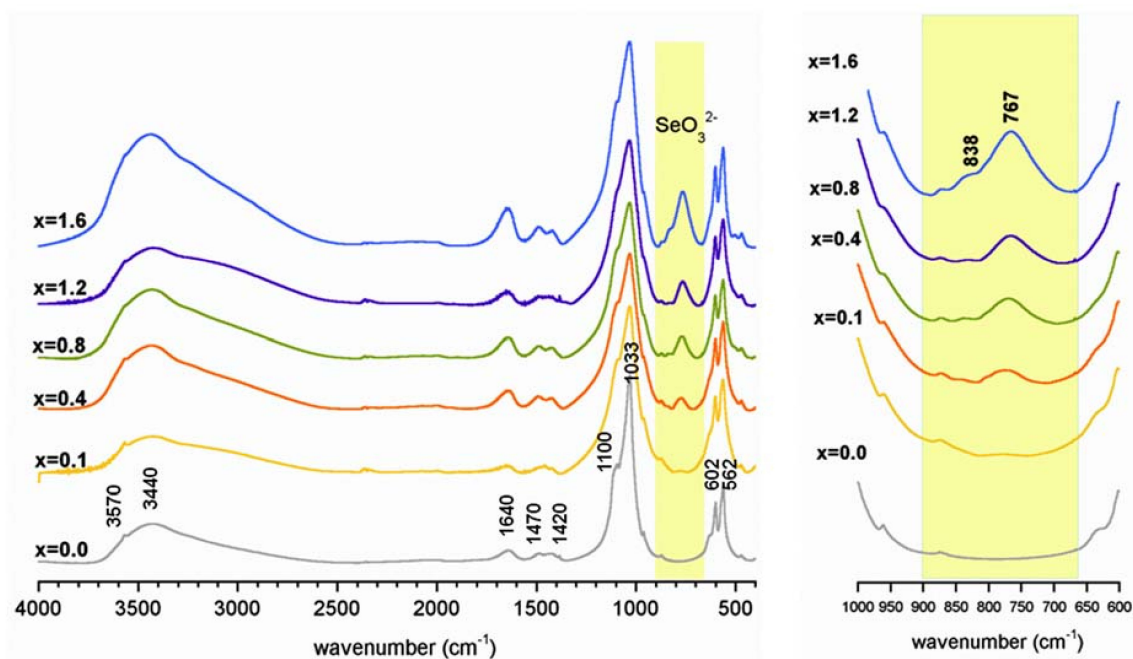


Figure 5. The FT-IR transmission spectra of the studied selenite-doped HA ($\text{HA}-x\text{SeO}_3$) and HA undoped. Reprinted from [43] with permission from Elsevier.

Wang et al. [46] recorded signals assigned to the phosphate and hydroxyl groups in the spectra, confirming that they were receiving SeO_3 -HA. The authors distinguished 1460 and 1420 cm^{-1} signals, indicating the presence of carbonates in the samples. Moreover, the signal at 784 cm^{-1} confirmed the incorporation of the SeO_3^{2-} ions, while the 874 cm^{-1} signal was assigned to selenite and carbonate ions. All bands derived from selenite and carbonate ions increased as the Se:P concentration increased.

In [38], the same bands were observed, but attention was paid to the significant reduction of the bands from the hydroxyl groups at 3570 and 630 cm^{-1} when compared with the pure HA spectrum.

Ma et al. [45] noted that, as the selenium ion content increased, there was a reduction in the strong bands attributed to the symmetrical and antisymmetric vibrations of the P–O bond in the $1200\text{--}900\text{ cm}^{-1}$ range, as well as in the bands of asymmetrical bending vibrations of the O–P–O bonds (bands at 604 and 567 cm^{-1}). They also noticed that, despite the incorporation of selenite ions, bands derived from phosphate groups were unaffected. However, the position of both the stretching and bending bands was obviously unaffected by the selenite substitutions. Similarly, when the Se:P ratio increased, the content of B-type carbonates decreased (bands at 1456 and 1413 cm^{-1}), while the intensity of the band at 1564 cm^{-1} , derived from A-type carbonates in the SeO_3 -HA samples, was higher than the intensity of the bands derived from B-type carbonates. The most intense bands from selenite appeared in the $900\text{--}800\text{ cm}^{-1}$ range, and originated from antisymmetric stretching vibrations, while the 766 cm^{-1} band originating from the bending vibrations of O–Se–O bonds increased as the Se concentration also increased.

On the recorded FT-IR spectra, Zhang et al. observed bands that were typical of HA, which originated from phosphates, carbonates, water and hydroxyl groups. They additionally recorded bands at 869 cm^{-1} and 775 cm^{-1} , which they assigned to the antisymmetric stretching vibrations of SeO_4 tetrahedron and bending vibrations (ν_3) O–Se–O [42].

In the studies [33,35,40], it was observed that the intensity of the band originating from the structural vibrations of OH groups at 3570 cm^{-1} decreased as the Se concentration increased. The authors [35] assigned the bands 767 and 712 cm^{-1} to the symmetric stretching vibrations (ν_3) of the selenite group. Moreover, when the concentration of selenite ions increased, the 856 and 823 cm^{-1} signals corresponding to the ν_1 and ν_3 vibrations of the SeO_3^{2-} group became stronger.

The spectra also showed bands derived from C–H, $-\text{CH}_2-$, and COO^- groups from oleic acid, which indicates that the samples were covered in this acid [35].

Liu et al. [40] observed a band originating from the vibrations of selenite groups at 766 cm^{-1} . This signal was observed for the first time with a sample containing a planned content of 3% Se, and it increased as the selenium content increased. There was also an increase in the intensity of the bands from CO_3^{2-} ions in the $1418\text{--}1566\text{ cm}^{-1}$ range, along with an increase in selenium content, which was probably caused by the facilitation of CO_3^{2-} group incorporation as the crystallinity of the samples decreased.

The examination of SeO_3 -HA coatings obtained by the PDL method confirmed the occurrence of HA-specific water bands, as well as phosphate and carbonate groups. It was also observed that, as the coatings increased in terms of Se content, not only did the bands from carbonates decrease, but the main band from phosphate groups (antisymmetric stretching) also decreased and its half-width increased. There was also a simultaneous loss of definition in the bending bands from the PO_4^{3-} group, as well as shift of the main phosphate band from 1040 cm^{-1} for the pure HA coating to about 1063 cm^{-1} for the coating containing the highest concentration of selenium. This band shift was caused by a change in the length of bonds, which was a consequence of the incorporation of Se ions [54].

3.4. Examinations Using the Raman Spectroscopy Method

Studies on samples of hydroxyapatites doped with selenium ions, conducted using Raman spectroscopy, have been described by several authors.

When comparing coatings with pure HA and SeO_3 -HA, obtained using the PLD method, in material doped with Se ions, signals that were characteristic of phosphates were confirmed: they were strongest at 960 cm^{-1} from bending vibrations (ν_1), at about 1070 cm^{-1} from stretching vibrations (ν_3), at 590 cm^{-1} from bending vibrations (ν_2), at 430 cm^{-1} from bending vibrations (ν_2) and symmetric stretching bands (ν_1) and at 1064 cm^{-1} derived from carbonate groups. In addition, a band of approximately 830 cm^{-1} originating from the symmetric stretching vibrations of the SeO_3^{2-} groups was registered for the SeO_3 -HA coatings [54].

Kolmas et al. recorded three bands at 911, 873, and 843 cm^{-1} for the sample doped with SeO_4^{2-} ions, which was assigned to selenate ions [43].

In contrast, Yilmaz et al. [55] examined the SeO_4 -HA coating using Raman spectroscopy. In addition to the bands characteristic of phosphates at 1072, 961, 590, and 429 cm^{-1} , as well as bands originating from the substrate at 629, 270, 209, and 136 cm^{-1} in a sample soaked for 14 days in a $1.5 \times \text{SBF}$ doped with Se ions, a weak signal from selenates was also observed at 764 cm^{-1} [55].

3.5. Examinations Using Nuclear Magnetic Resonance Spectroscopy (NMR)

Kolmas et al. [41] subjected the obtained samples of HA doped with SeO_3^{2-} and SeO_4^{2-} selenium ions to ssNMR examination. Spectra were recorded for the nuclei ^{31}P , ^1H , and ^{77}Se .

The ^{31}P spectra were recorded using two techniques: one-pulse (Bloch-decay, BD) and $1\text{H} \rightarrow 31\text{P}$ cross-polarization (CP). One signal characteristic of hydroxyapatite samples at about 3 ppm was recorded on the spectra. It was noted that the signal in the ^{31}P BD NMR spectra was narrowest for the pure HA sample and widest for the SeO_3 -HA sample; moreover, because the line width was correlated with the crystallinity of the sample, this was indicative of its reduction in the selenium-doped samples.

The signal in the ^{31}P CP NMR spectra was deconvoluted into two components: narrow, derived from phosphate groups inside the crystal located near the protons of structural hydroxyl groups, and broad, derived from phosphate groups located near to the water-rich environment (especially from phosphors from the hydrated surface layer) [41,57]. The wide component was relatively the most intense in the spectrum of the sample containing SeO_3^{2-} , indicating a more extensive hydrated surface layer and, thus, a more developed surface. In all of the recorded ^1H MAS NMR spectra, two signals can be distinguished at about 0 and 5.4 ppm. The signal at about 0 ppm comes from structural hydroxyl groups. The OH groups form columns in the channels, but they are so far apart that it is impossible to create hydrogen bonds between them. In spectra registered for samples doped with selenium, the chemical shift of this signal moved toward positive values, which were highest in the sample containing selenites. The widening of the signal in the Se-HA samples was also observed to be more significant than the signal from pure HA. The authors suggest that, due to the incorporation of selenium ions into the hydroxyapatite structure, the column structure of the OH group became disturbed, resulting in the formation of weak hydrogen bonds. The content of structural hydroxyl groups was calculated, and it was observed that there was a significant loss of HA crystals with incorporated selenite ions (49% OH groups relative to stoichiometric HA) and selenate (63%) when compared to pure HA (77%). It was also proven that this reduction in the content of structural hydroxyl groups was related to the decreased size of the crystals [43,57] due to the absorption of water from the crystals' surface into the columns of the hydroxyl groups. Thus, the authors postulate that the reason for the loss of structural hydroxyl groups is two-fold: not only are OH groups removed during substitution, but also water is present in the OH columns [41].

The signal at about 5.4 ppm is attributed to water adsorbed onto the surface of the crystals. The spectra for the ^{77}Se nucleus were recorded using the $^1\text{H} \rightarrow ^{77}\text{Se}$ CP technique. The obtained spectra for the HA containing selenates showed two intense signals at 1045 ppm, and a much weaker one at 1027 ppm. Furthermore, for the sample containing selenites, there was an analogously strong signal at 1310 ppm and a weaker one at 1325 ppm. It was proven that the strong signals on both spectra originated from selenium ions embedded in the crystalline lattice of the crystal, while the lower intensity signals were derived from selenates and selenites in the hydrated surface layer.

4. Biological Examinations of Hydroxyapatite Materials Doped with Selenium

Biological examinations of HA materials containing selenium have focused primarily on the determination of their antibacterial, anticancer, and cytotoxic effects, as well as their influence on the development of bone tissue. In addition to in vitro studies, some research groups have also

conducted experiments using an in vivo animal model (mice or rats). Below, we will try to illuminate the results and conclusions from the studies.

4.1. Antibacterial Activity

Studies to determine the antibacterial activity of SeO_3 -HA and SeO_4 -HA were carried out on the following bacterial strains: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Salmonella enteritidis*. This is because they are the bacteria that contribute most often to infections during orthopaedic surgery. *S. aureus* is of particular note, as it is a very common pathogen that causes infections during bone implantation due to the ease of biofilm formation and the presence of numerous antibiotic-resistant strains.

Measurements of antibacterial activity have typically been performed through determining the optical density of the bacterial suspension, counting the CFUs attached to the material, determining the area of inhibition using the disk diffusion method, or evaluating biofilm formation. The measurements were taken after the bacteria had been incubating for an appropriate length of time on the growth medium in the presence of the examined materials.

In the case of *S. aureus*, all of the studies [47,53,54,58,59] observed that SeO_3 -HA materials significantly reduced bacterial viability, inhibiting both their growth and biofilm formation, when compared to the control and samples with pure HA. According to the study by Rodríguez-Valencia [54], the introduction of 0.6 wt % of selenium ions into the HA structure was already sufficient to inhibit the formation of an *S. aureus* biofilm (as well as *P. aeruginosa*) (Figure 6).

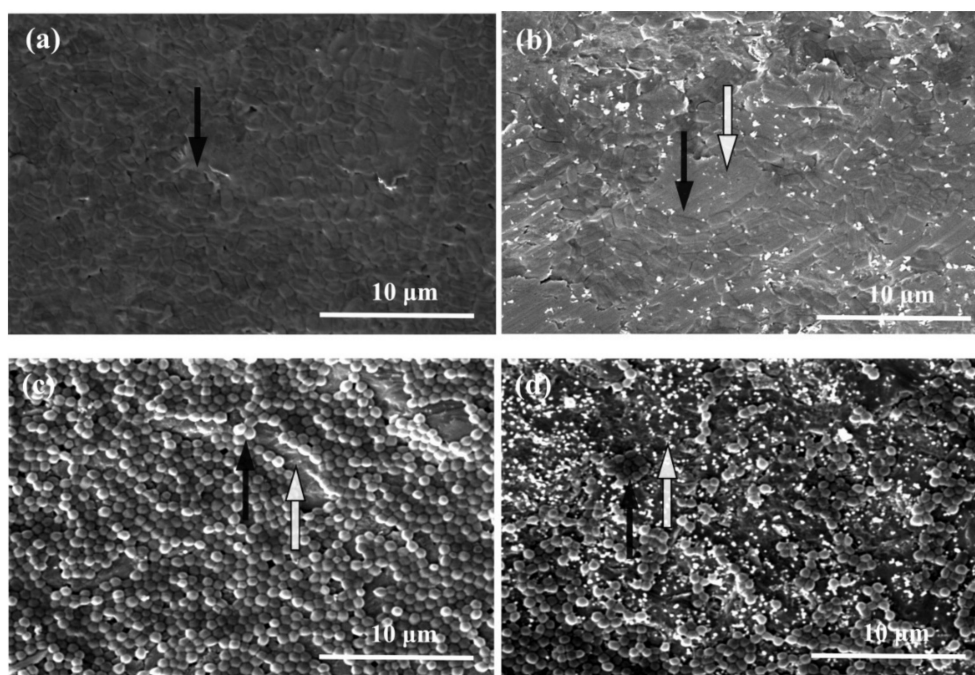


Figure 6. SEM micrographs of the aspect of the biofilm after inoculating *P. aeruginosa* (a and b) and *S. aureus* (c and d) on the RHA2.5Se (b and d) coatings compared to HA (a and c). White arrows indicate non-colonized areas and black ones indicate the shape of an exemplar of that bacterium. Reprinted from [54] with permission from John Wiley and Sons.

Additionally, according to Uskokovic [47], despite the antibacterial effect of the SeO_3 -HA material on *S. aureus*, these properties were lost over time and the bacterial population returned to a level similar to that observed in the negative control group. This may indicate that those *S. aureus* bacteria that survived their initial exposure to SeO_3 -HA did not later show susceptibility to SeO_3^{2-} ions, which allowed the population to rebuild.

The results of studies carried out on *E. coli* bacteria are interesting. These bacteria are characterized by their ability to reduce SeO_3^{2-} ions to Se^0 , which they can then use to synthesize the amino acids selenocysteine and selenomethionine. Due to this fact, the level of SeO_3^{2-} ion concentration will determine whether *E. coli* growth is stimulated or inhibited. According to the study carried out by Kolmas [58], a selenium content of 3.6 wt % in the HA structure was insufficient to inhibit the growth of *E. coli*. In turn, according to Murugan [59], there was a decrease in the functioning and lifespan of bacteria at all applied concentrations of SeO_3 -HA, which was further confirmed by the study of Uskokovic [47], in which *E. coli* bacteria were still sensitive to the examined biomaterial 72 h after the start of incubation at the highest level of selenium content in the analysed SeO_3 -HA material (1.92 wt % and 3 wt %).

It is worth emphasizing that studies performed on both *E. coli* and *S. aureus* [47,59] indicate that the SeO_3 -HA material has a stronger effect on these strains than on the first of the mentioned strains. This may be due to the presence of a thinner layer of peptidoglycan in the cell wall in Gram-negative bacteria, which makes it easier for the active substance to penetrate inside the cell.

According to Rodríguez-Valencia [53], as the selenium content in SeO_3 -HA increased, there was a gradual increase in its antibacterial activity against *S. epidermidis* and a gradual inhibition of biofilm formation until it completely disappeared 72 h after the start of incubation. This was confirmed by the study of Yilmaz [55], in which a significant decrease in optical density was observed after 72 h of incubation in a sample with an SeO_4 -HA-coated titanium implant, as compared with a sample with an uncoated implant, one with a pure HA coated implant and the control sample.

Antimicrobial activity was also tested for the strains *P. aeruginosa* and *S. enteritidis*. The results of the studies confirmed the effectiveness of SeO_3 -HA in inhibiting the growth of these microorganisms by [47,54].

In conclusion, the studies carried out confirmed that HA material containing selenium has an antibacterial effect on individual bacterial strains. The antibacterial effect of this biomaterial is based on inducing the formation of reactive oxygen species in a bacterial cell. According to Kramer and Seko [60,61], oxidation and reduction reactions which occur during selenium metabolism lead to the formation of hydrogen peroxide and superoxide (i.e., reactive oxygen species), which, in turn, contribute to oxidative stress and damage to the bacterial cell wall. It is worth noting that the antibacterial effect of SeO_3 -HA is stronger for Gram-negative bacteria than it is for Gram-positive ones [47,59], while the adsorption of antibiotics on the surface of SeO_3 -HA (e.g., vancomycin) increases the obtained antibacterial properties [47].

4.2. Anticancer, Cytotoxic, and Osteoinductive Effects

The main assumption during the synthesis of hydroxyapatite materials doped with selenium is that biomaterial will be obtained that will effectively inhibit the development of bone cancers, while showing no cytotoxic activity on healthy tissue. Another important feature of this material is its ability to induce osteogenesis in order to stimulate bone tissue reconstruction at the site of a bone defect.

In vitro studies were performed on healthy mouse cells (mouse MC3T3-E1 preosteoblasts and mouse fibroblasts collected from the lungs), healthy human cells (human foetal hFOB osteoblasts, bone marrow stromal cells (BMSC) and human fibroblasts), mouse cancer cells (murine ATCC osteosarcoma cells and K7M2), and human cancer cells (human MG-63 osteosarcoma cells, Saos-2, and MNNG/HOS). In vivo studies were performed on Wistar rats and BALB/c mice.

4.2.1. In Vitro Studies

In vitro tests on healthy cells primarily allowed for an evaluation of the biocompatibility of the discussed material, as well as its osteogenic properties. A study on biocomposites constructed from SeO_3 -HA and silk fibrin (SF/Se-HA), carried out by Wang [34] on BMSC cells, clearly indicated that the presence of selenium in the biomaterial increased the intensity of cell population growth on

days one to seven from the start of the study compared to the control group and SF/HA sample, in which a relatively slow increase in cell population growth was observed. In addition, after seven days of incubation, the observed cell density was highest for the biomaterial samples with the highest selenium content. An increase in selenium content resulted in an increase in the rate of osteoblast proliferation, which was additionally confirmed by Vekariya's study [62]. In turn, the study on the effect of SeO_3 -HA materials on MC3T3-E1 cells, carried out by Jianpeng [35], allowed for an assessment of cytotoxicity with respect to healthy cells. They analysed the effect of two SeO_3 -HA materials with a ratio of $\text{Se}:(\text{P} + \text{Se})$ of 0.05 and 0.1, respectively (using different concentrations of the examined materials). In this case, not only was no cytotoxic activity observed in the sample containing the material with the highest selenium concentration, but it was also found to have a greater beneficial effect on the viability of MC3T3-E1 cells than in the case of pure HA samples. Rodríguez-Valencia's studies [53,54] confirmed the lack of cytotoxic activity of SeO_3 -HA, where cell viability in the samples containing SeO_3 -HA was at a similar or even a higher level than in the pure HA samples. The morphology of the MC3T3-E1 cells also did not change with incubation time [35,54]; numerous filopodia and lamellipodia could be observed in the cell structure, which enabled them to spread on the surface of the biomaterial [54]. This confirmed the biocompatibility of SeO_3 -HA material with mouse preosteoblasts cells. The activity of osteoblasts was also analysed in studies carried out by Rodríguez-Valencia [53] and Uskokovic [47]. It was shown that osteoblastic activity also increased with incubation time and with increased selenium content in HA materials. Differences in the results were due to the selenium concentrations in the HA material, where osteoblastic activity was highest at higher selenium concentrations. For the first study, this was observed for sample containing the highest concentration (2.7 wt %) of selenium in the HA, while in the second study, this activity was highest at a concentration of 1.23 wt % and decreased when the selenium content increased any higher than this value. At the highest selenium concentration used (3 wt %), osteoblastic activity was significantly inhibited when compared with the pure HA sample, abnormalities in the cells' structure were visible and some of them died within the first 24 h of incubation. This was due to the effect of selenium on ROS generation and, thus, on the level of oxidative stress in osteoblasts. A suitable amount of selenium enabled the maintenance of oxidative stress at a level where it was able to exert a beneficial effect on osteoblastic activity; however, too much of this element had the effect of increasing oxidative stress to a level at which apoptotic pathways were activated [63–66].

In vitro tests carried out on cancer cells facilitated the determination of the anticancer efficacy of the examined biomaterials. In virtually every case, these tests were carried out in parallel with studies on healthy cells, which led to a fuller picture of the biomaterial activity. Studies conducted by Wang [34,46] and Uskokovic [47] showed that SeO_3 -HA material had no effect on the viability and proliferation of healthy cells, while it inhibited the growth and reduced the viability of cancer cells (compared to the control group or HA sample). The effect of SeO_3 -HA on osteosarcoma cells was stronger at higher selenium levels. The studies of Kolmas [36] and Yilmaz [55] confirmed the abovementioned effect of SeO_3 -HA (and SeO_4 -HA) on cancer cells; however, their results also indicated inhibition of growth and the reduction of viability of healthy cells. According to Kolmas [36], this was due to the so-called "burst release" of selenium ions from the biomaterial surface during the first 10 h following the beginning of the study, leading to a high concentration of selenium ions and, thus, causing the effective reduction of viability of both cancer cells and healthy osteoblasts.

Wang [46] confirmed that the anticancer effect of SeO_3 -HA is due to the induction of apoptosis in cancer cells. In order to illuminate the mechanism of its anticancer activity, both Wang [46] and Wang [37] (convergence of names) performed a number of studies. They both measured the fluorescence intensity of the ROS marker to determine whether SeO_3 -HA causes an increase in the amount of reactive oxygen species generated. The results of both studies confirmed the above dependence. In other words, they found that Se-HA enhanced the formation of intracellular ROS, which in turn induced apoptosis and activated caspases. Caspase-3 is a key factor in the apoptosis process and is activated by caspase-8 and caspase-9, which initiate two different apoptotic pathways:

the caspase-8 extrinsic and the caspase-9 intrinsic apoptotic pathways. In his study, Wang [37] confirmed the presence of activated caspase-3 and caspase-8 and caspase-9 in samples with a high selenium content (not only in the sample with Se-HA, but also in the one with Na_2SeO_3), which clearly indicated that that caspase-dependent apoptosis was associated with the presence of high selenium content (see Figure 7).

Finally, it is worth mentioning the study performed by Kolmas [43], which tested the toxicity of HA material doped with SeO_3^{2-} or SeO_4^{2-} ions in *Vibrio fischeri* bacteria and *Spirostomum ambiguum* protozoa. The results indicated that SeO_3^{2-} ions were toxic to a certain degree for both microorganisms, while this effect was not visible in the case of SeO_4^{2-} ions, which may indicate that selenate ions (VI) are less toxic than selenite ions (IV).

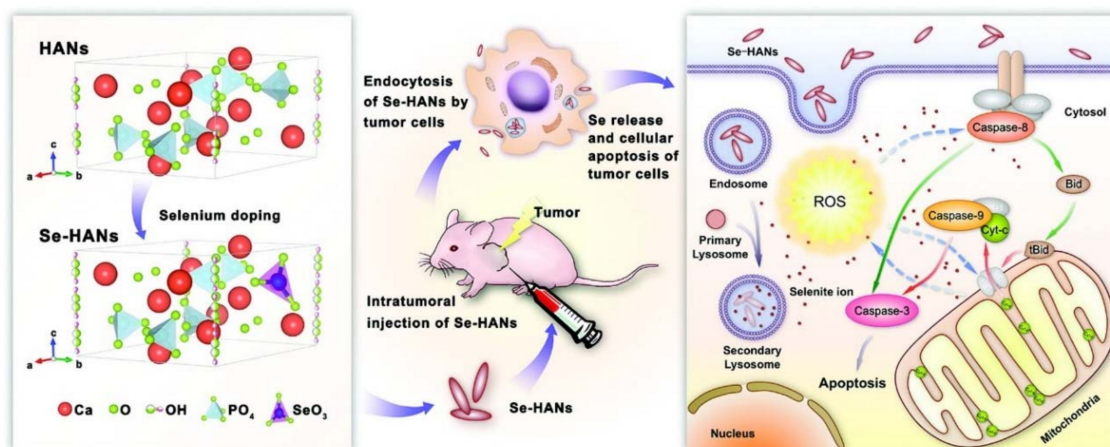


Figure 7. Preparation and working principle of antitumor nanoparticles. Se-HANs were fabricated using selenite to replace phosphate of HANs. Intratumoral injection of Se-HANs was performed on the xenograft osteosarcoma model. Se-HANs were internalized into tumor cells by nonspecific endocytosis and rapidly degraded in the acidic lysosome to release selenium. Cell apoptosis was activated by a primary, selenium-induced, caspase-dependent apoptosis pathway synergistically orchestrated with the ROS generation. Reprinted from [37] with permission. Copyright (2016) American Chemical Society.

4.2.2. In Vivo Studies

In in vivo studies on Wistar rats, Wang [44] applied the examined biomaterials in previously prepared skull bone defects. These materials were a biocomposite made of hydroxyapatite and chitosan (HA/Ch) and the biocomposite SeO_3 -HA/Ch. The control group was made up of rats with a cranial bone defect, and no biomaterials were applied in this group. No complications were observed with the graft, and all of the rats survived until the day of euthanasia (which took place four, eight, or 12 weeks after implantation). The histological examination showed that there was inflammation within the graft or necrosis of the tissue, which was indicative of the biocompatibility of the material with the tissue (this was due, inter alia, to the high porosity of the material). The scaffolding created by the implant began to partially degrade eight weeks after implantation. It was replaced by newly-formed bone tissue, which, however, was less abundant than in the control group. Twelve weeks after implantation, an increasing number of bone connections between the host bone and the implant could be observed in groups with SeO_3 -HA/Ch and HA/Ch biomaterials. In addition, in the case of SeO_3 -HA/Ch, larger quantities of stromal cells, ossein, and fibrous cartilage cells were visible within the defect. Furthermore, it should be noted that characteristic polymorphonuclear cells were present, which supported the formation of new bones in the sample with SeO_3 -HA/Ch [67]. In addition, for the SeO_3 -HA/Ch group, the formation of new blood vessels was visible (neovascularization). The determination of calcein in all of the implantable materials tested was performed 12 weeks after

implantation. The introduction of selenium into the structure resulted in the recruitment of progenitor cells and osteoclasts, which had a positive effect on scaffold degradation and bone remodelling. However, it is difficult to obtain a complete bone-width between the material and the host bone by osteogenesis and neovascularization. According to [68,69], a significant amount of time is needed to bind HA to the host bone, so more research is needed to confirm this. Basing their results on the number of bone beams, Wang [37] also stated that bone quality was higher in the SeO_3 -HA/Ch group, since this parameter was highest in this sample.

In turn, Wang [37] carried out an *in vivo* study on osteosarcoma cells in BALB/c mice. In addition to applying SeO_3 -HA materials with different levels of selenium content, control groups were prepared, which were HA solutions with various amounts of Na_2SeO_3 added. For the sample of SeO_3 -HA material with the highest selenium content, the author found that cancer growth was inhibited and the volume and weight of tumours were reduced. The control group sample with an identical selenium concentration gave a similar result. Although all of the mice survived until the end of the experiment without any visible changes in body weight, the analysis of various biochemical parameters (AST, BUN, CREA, and LDH) indicated differences between the various groups in terms of systemic toxicity. It should be taken into account that *in vivo* toxicity may have decreased under the influence of selenium ions by inducing apoptosis in cancer cells, impairing the metabolism of these cells and protecting healthy tissues. On the other hand, too high a dose of selenium may cause side effects in terms of organ function in the examined mice, thus leading to increased toxicity. The toxicity level was lowest in the SeO_3 -HA group with the highest selenium content, which was indicated by significantly reduced biochemical parameters. This may be explained by the gradual release of selenium from the SeO_3 -HA material, which was additionally regulated by the acidic pH of the cancer tissue environment. Therefore, the concentration of selenium in the tumour was high, with a small quantity of selenium ions leaking out of the cancerous tissue. For the control group, where Na_2SeO_3 was mixed with HA, the whole substance immediately decomposed and spread beyond the cancer tissue into healthy tissues, which caused an increase in toxicity. Studies on healthy BALB/c mice confirmed the lack of toxicity of SeO_3 -HA materials in comparison with the HA/ Na_2SeO_3 control sample, which showed a significant increase in systemic toxicity. This means that the use of SeO_3 -HA material enables the avoidance of selenium toxicity because it is gradually released from the material. Wang [37] also confirmed that the mechanism of the activity of SeO_3 -HA materials in the *in vivo* model was analogous to that presented in *in vitro* studies.

Finally, it is worth mentioning that Wang [38] also showed the beneficial anticancer effects of SeO_3 -HA material on hepatocellular carcinoma cells in a study on BALB/c mice, which increased the survival of the mice and improved their biochemical parameters.

5. Conclusions and Future Perspectives

Hydroxyapatite that contains selenium ions is a material with high application potential. The studies presented in this review have demonstrated that it is possible to introduce both IV and VI selenium ions into the interior of the crystals, preserving the hexagonal structure of the HA. The obtained crystals are nanocrystalline, with a strongly-developed specific surface and a tendency to form large agglomerations. IV and VI selenite ions can also be located in the hydrated surface layer and strongly adsorb onto the surface of HA crystals. Se-HA was successfully used to form composites with silk fibres, sodium alginate and lysozyme. The studies demonstrated that it has the ability to cover metallic implants. Biological tests confirmed the antibacterial activity of SeO_3 -HA materials; however, it had a stronger effect on Gram-negative strains than Gram-positive ones. *In vitro* studies indicated the beneficial effect of apatite materials containing selenium on osteoblastic activity. However, it is worth noting that high concentrations of selenium caused a significant increase in the toxicity levels of normal cells. *In vitro* cancer cell research, supported by *in vivo* tests, clearly confirmed the inhibition of growth and the reduction of vitality.

These promising results obtained for hydroxyapatite materials containing selenium mean that the subject is still relevant. It is possible to find some attempts in the literature to synthesize co-substituted hydroxyapatites, e.g., those containing selenium IV ions along with Mn^{2+} manganese, Zn^{2+} zinc or Fe^{3+} iron ions [59,70,71]. There have also been attempts to use Se-HA to create systems for the delivery of medicinal substances to bone tissue, e.g., antiretroviral drugs (from the group of bisphosphonates) or antibiotics [36].

The future steps of the studies on Se-HA materials should be focused on appropriate selenium concentration causing low toxicity towards normal cells and sufficient toxicity towards cancer cells. Moreover, the physicochemical studies on selenium-doped materials should be continued. For example, thermal stability of Se-HA is not yet analysed. Next, bioceramics based on Se-HA materials should be developed.

The future research should also focus on multifunctional composite materials containing selenium-doped HA which could be used as drug delivery systems.

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Conflicts of Interest: The authors declare no conflict of interest.

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