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Immobilization of Biomolecules on Hydroxyapatite and Its Composites in Biosensor Application: A Review

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Abstract: Hydroxyapatite (HAp) is a calcium phosphate ceramic biomaterial in biomedical devices. The composited HAp nanoparticles had better clinical performance than micro size and without composites. In addition, it makes it easier for researchers on the electrode surface to design biosensor fabrications because of the large surface area of HAp and facilitating the adsorption of biomolecules. The immobilization of biomolecules on hydroxyapatite and its composites showed an increase in stability and durability for a long period of time due to the high affinity of HAp. This review describes several biomolecule immobilization techniques on hydroxyapatite and its composites in biosensor applications.

Keywords: biomolecules; biosensor; hydroxyapatite; composited hydroxyapatite; immobilization.

1. Introduction

Researchers develop applications of biomaterials in the treatment of diseases and improve the properties of biomedical. Hydroxyapatite, HAp ($Ca_{10}(PO_4)_6(OH)_2$), has attracted the attention of many researchers in the last decade and plays an important role in biomedical applications due to its chemical composition in particular calcium and phosphorus, which is similar to the composition of human bones and teeth [1]. In addition, HAp has a very good affinity for biomolecules. Many studies on HAp found that HAp is formed into various structures and sizes, and various nano-sized HAp synthesis methods have attracted much attention [2].

In the last decade, hydroxyapatite has been used in various electrode surface modification strategies and techniques in biosensor applications. Biosensors have experienced growth and development in research abroad over the last two decades as analytical devices for converting biological responses into measurable signals due to their easy and portable use.

Currently, several studies of hydroxyapatite in biosensor applications are a component of the immobilization matrix of biomolecules as bioreceptors such as proteins, aptamers, and DNA [3]. Biomolecules are biological molecules in living organisms, including the proteins, carbohydrates, fats, and nucleic acids that build cells. Immobilization is an important step in the performance of biosensors in maintaining the conformation and biological activity of biomolecules, as well as ensuring the accessibility of bioreceptors' active sites on the target analyte. This review aims to review the use and development of hydroxyapatite and also discuss

the description of several biomolecular immobilization techniques on hydroxyapatite and its composites in biosensor applications.

2. Hydroxyapatite and its Composites

Apatite (Ap) is a natural inorganic material that is used as an important mineral component in the bones and teeth of humans and animals [4,5]. Hydroxyapatite (HAp) ia a ceramic biomaterial that has a calcium phosphate composition (CaP) with the theoretical formula (Ca10(PO4)6(OH)2), which is bioactive with the ability to form direct bonds with living tissue [2,6]. Minerals used as constituents of HAp can be obtained from raw materials in the form of calcium oxide as a source of Ca which is reacted with diammonium hydrogen phosphate as a source of phosphate [7]. HAp has distinctive characteristics, such as high porosity and the ability to exchange ions, so it has the potential as a base material for sensors [8].

HAp has attracted a lot of attention from researchers because of its wide application, such as bone and dental implants, protein separation, absorbent, and immunosensor. It has higher stability than other types of CaP crystalline phases [3,9]. Such as monocalcium phosphate monohydrate (MCPM), dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), α-Tricalcium phosphate (α-TCP), β-Tricalcium phosphate (β-TCP), amorphous calcium phosphate (ACP), fluorapatite (FA), oxyapatite (OA), tetra calcium phosphate (TTC), and calcium deficiency hydroxyapatite (CDHAp) or compared to liposomes and micelles which tend to disappear under certain conditions, has a high affinity, and multi-adsorbing sites [6,10]. The hexagonal crystal structure of HAp consists of a PO_4 tetrahedral with Ca^{2+} and OH^- ions surrounding it, as shown in Figure 1, with a unit cell of HAp includes ten calcium ions (40% Ca), six phosphate ions (18,5% P), and two hydroxyl ions (3,38% OH radicals) by weight with 1,67 Ca/P molar ratio and also cell parameters a = b = 9.4225 Å and c = 6,8850 Å [10–12].

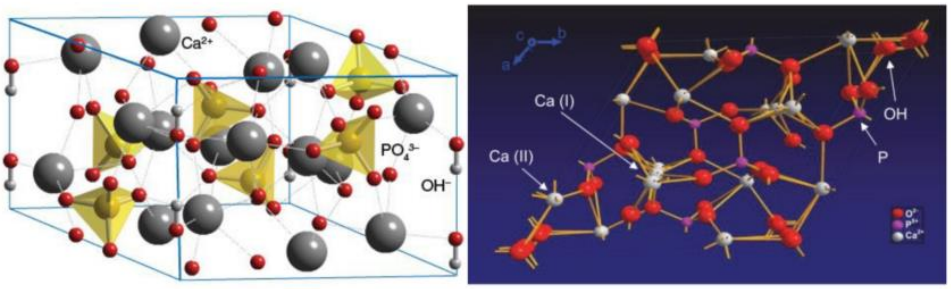

Figure 1. The crystal structure of hydroxyapatite. From ref [10] with CC BY license.

HAp has advantages such as low cost, mild synthesis, and ease of function when compared with noble metal nanoparticles such as Pd, Au, Ag, Pt, Os, Ir, Rh, and Ru [13,14]. HAp nanoparticles are more desirable for better application in various fields because they have a larger surface area. Still, in electrochemical devices, HAp nanoparticles have low conductivity and are easy to agglomerate, and they can not be used as a single biomaterial [15,16]. Therefore they need to be doped by metal ions such as gold and silver; carbon nanoparticles such as carbon nanotube and graphene; or other conductive materials such as bioceramic ZrO2 and TiO2, chitosan, or mineral/fibers to form composites with HAp as the main constituent of composites [13,15,17,18].

Composites are used to improve the properties of HAp by increasing their electron conductivity to improve the current response and immobilization of biomolecules on the

electrode surface. In addition, the multi-adsorbing sites of HAp were successfully applied to electrochemistry without the addition of ions for electroanalysis [15] because HAp has a unique 3D porous network structure and excellent adsorption as a reagent for removing heavy metal ions and organic molecules and has an isoelectric point (IEP) at pH 7.3 [19]. Based on Table 1, when viewed from the limit of detection value, shows that a better composite for hydroxyapatite is gold nanoparticles because it is a noble metal nanoparticle with excellent electrical conductivity from its electrons and increasing the available surface area for immobilization biomolecules [20,21]. Metal nanoparticles have apprehended incredible guarantees and are broadly applied in the biomedical field because large surface area, high reactivity to live cells, small sizes, and stability over a high-temperature range [22,23].

Preparation processes and suitable synthesis methods for preparing nano-sized HAp and its composite materials have been continuing to be researchers for many years. Nano-sized HAp can increase the efficiency of biomolecule immobilization due to increased adsorption, so the sensor system is more sensitive [2,4]. The presence of nanomaterials in the composites made a broad interfacial area and can be considered the most adaptable materials in the present day [24]. In 2011, HAp was synthesized using chemical precipitation composited with Prussian Blue (PB) and incubated with horse radish peroxidase (HRP) and secondary anti-AFP antibody (Ab2) to create electrochemical immunosensor labels to improve sensor performance analysis. The TEM results found that the nano-HAp was rod-shaped with a size of 20 nm, and when PB was composited, it showed that the HAp matrix could strongly bind homogeneous PB [14].

The same method was used in making HAp composites with colloidal gold nanoparticles and chitosan to detect prostate-specific antigen (PSA). The TEM results showed that the HAp nanocrystals were well dispersed with a size of 50 nm as a matrix to hold the immobilized antibody accelerated binding, which was more accessible to the antigen. They explained that the GNP layers of 10, 16, and 30 nm sizes could be strongly absorbed on the surface of HAp, forming a uniform porous structure, which promotes much better immobilization of biomolecules due to the formation of a structured film and an increase in the biocompatible area for biomolecule adsorption. In addition, with the addition of chitosan, anti-PSA antibody biomolecules retain their bioactivity after binding to the HAp/GNP surface [25].

In addition, in 2019, the synthesis of nano-HAp used a hydrothermal method with varying pH, which would be composited with gold nanoparticles and reduced graphene oxide to detect urea acid. SEM results showed that a pH of 4.5 produced nano-HAp in the form of nanowires with a size of 10-15 nm, which were distributed homogeneously compared to pH of 3.5; 4; and 5.5, and the TEM results showed that the AuNP were evenly distributed in the HAp/rGO composite due to the large specific surface area of HAp and the better film-forming ability of AuNP [26].

3. Biosensor

Biosensors provide an attractive means as analytical devices in converting biological responses into measurable signals generated by chemical reactions. Easy use, cheap, simple, small, portable, and can be used by semi-skilled operators has become a public interest in developing biosensors [27]. Biosensors' high sensitivity and fast response time solve analytical challenges of various common detection methods [28,29]. Biosensors are combined with various nanomaterials such as carbon nanotubes, graphene nanosheets, metal nanoparticles, metal oxide nanoparticles, or nanoconjugates to improve the performance of the biosensor in amplifying signals, increasing surface area, and as stabilizers for biological receptors [30].

Biosensors generally have three important elements, such as bioreceptors, transducers, and signal reading devices, as shown in Figure 2 [31,32].

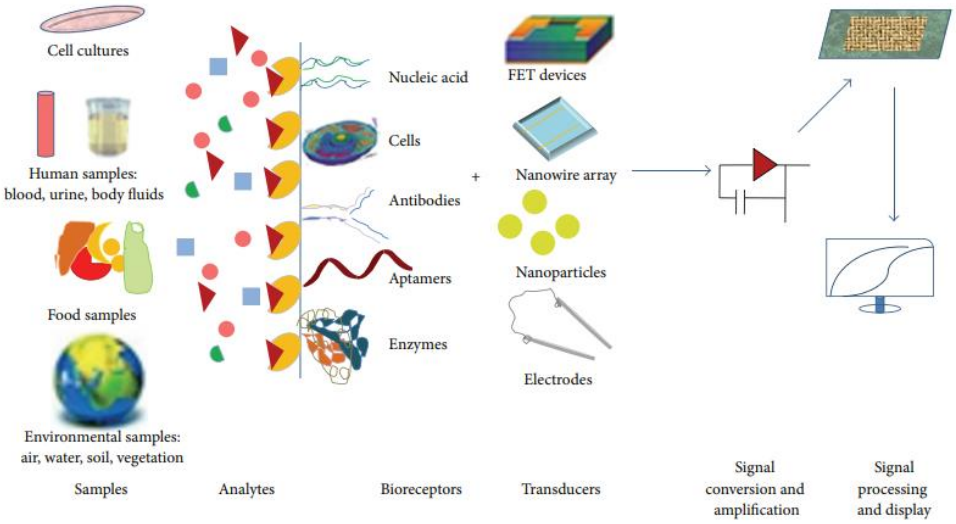

Figure 2. The schematic diagram shows the components of the biosensor. From ref [33] with Creative Commons Attribution License.

The surface of the electrode where the sensor process is modified using a base material such as nanoparticles, then the bioreceptors are immobilized on the base material with different techniques. The bioreceptors specifically and selectively bind to the target analyte, causing a signal that is detected by the transducer in the form of a biological signal of the altered bioreceptor-analyte interaction which is converted into an electronic signal and sent for processing using software that will give it a physical form. Biosensors are highly selective for specific interactions between biological receptors on the sensor with a specific binding affinity for the desired molecule [34].

Biological receptor elements (bioreceptors) are the main components in the construction of biosensors, and bioreceptors commonly used in biosensors are protein biomolecules such as enzymes, antigens, antibodies, nucleic acid biomolecules such as DNA and aptamers, or whole cells. Currently, thousands of DNA and RNA aptamers have been applied to detect targets such as proteins, peptides, amino acids, antibiotics, small chemicals, viruses, whole or part of cells, and even metal ions with high affinity and specificity. Aptamers are also applied mainly for diagnosis, treatment, biosensors, and bioanalytics [35]. Although enzyme-based biosensors have been the most commonly used over the last two decades, antibody-antigen-based biosensors (immunosensors) have high specificity and sensitivity, and nucleic acid or aptamer-based biosensors (aptasensors) have high specificity for analytes containing nucleic acids and microbial strains [36].

4. Immobilization of Biomolecules on Hydroxyapatite and Its Composites

Biomolecules are biological molecules in living organisms, including proteins, carbohydrates, fats, and nucleic acids forming cells. Proteins are multifunctional heteropolymer macromolecules containing natural amino acids linked by peptide bonds determined by the nucleotide sequence in genes. Proteins depend on the amino acid sequence that determines their folding, function, rigidity, stability, flexibility, and interaction with their environment so that proteins are folded into a three-dimensional structure that allows them to interact with other molecules and carry out their functions [37]. Proteins can be enzymes, antigens, and antibodies.

In contrast, nucleic acids are macromolecules in cells in the form of linear polymers composed of nucleotide monomers bonded through phosphodiester bonds such as DNA, RNA, and aptamers.

Immobilization is a key step in the performance of biosensors in maintaining the conformation and biological activity of biomolecules, as well as ensuring the accessibility of bioreceptor active sites on the target analyte [38,39]. Immobilization is a very attractive technique, especially in clinical applications, because it is simple, precise, and allows direct analyte detection [40]. Some of the methods used to immobilize biomolecules as bioreceptors in biosensors include as shown in Figure 3 [38,41].

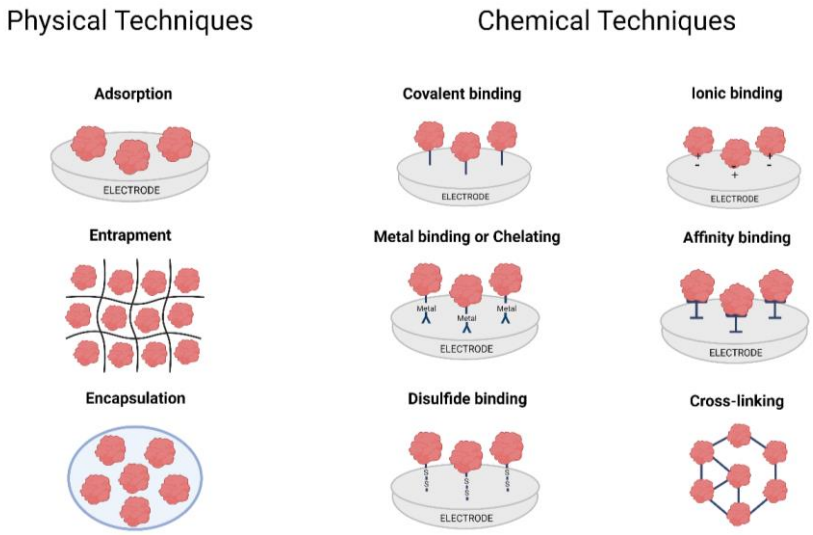

Figure 3. Biomolecules immobilization technique on the electrode surface.

4.1. Physical adsorption.

Macromolecules such as proteins are able to be physically adsorbed on the surface of matrices such as cellulose, silica gel, glass, hydroxyapatite, collagen, polystyrene, and graphite [38]. Adsorption is the simplest and fastest immobilization technique that involves binding biomolecules to the electrode surface through weak non-covalent interactions such as hydrogen bonds, van der Waal interactions, and hydrophobic interactions [42]. However, this technique is susceptible to temperature and pH [43]. In 2019, the UAO/HAp-rGO/AuNP biosensor was developed to detect urea acid. The immobilization of the uricase enzyme (UAO) as a bioreceptor is adsorbed on the matrix surface due to the large surface area on the HAp nanowires, thereby holding the enzyme bioreceptor immobilized resulting in a binding site that is more accessible to the target urea acid [26]. In addition, in 2009, a HAp/GNP biosensor was developed to detect hemoglobin. Immobilized hemoglobin is strongly adsorbed on the surface of HAp/GNP due to the porous structure of HAp, which increases protein loading [44].

4.2. Cross-linking.

Cross-linking is a technique that uses a crosslinker agent such as glutaraldehyde (which reacts with amino groups in protein), as shown in Figure 4. However, glutaraldehyde can inactivate some enzymes, so other alternatives are using polysaccharide dextran, bis-diaminobenzidine, diazonium salts, and functionally inert proteins such as bovine serum albumin (BSA) [43]. Glutaraldehyde is a bifunctional reagent with an aldehyde (-CHO) at each end that can react with two primary amine groups. One glutaraldehyde molecule bridges two

primary amine groups, ideally one from the biomolecule and the other from the solid surface of the matrix [45].

Biomolecules are ionically adsorbed on activated surfaces with positively charged primary amine groups and preparations that do not cross-slide covalently with glutaraldehyde [46]. In 2016, a JF collagen/HAp-NP/TBA biosensor was developed to detect thrombin. Immobilization of JF collagen/HAp-NP with TBA (thrombin binding aptamer) through a glutaraldehyde linker, where one side of the carboxylate of glutaraldehyde will bind to the amine on JF collagen and the carboxylate, on the other hand, will bind to TBA [3].

In addition, in 2011, a thionine/graphene sheet (GS-TH) biosensor with a Prussian Blue/HAp-HRP-anti-AFP label was developed to detect AFP (α-fetoprotein). Anti-AFP antibody immobilized on GS-TH via glutaraldehyde linker and then washed using BSA to block non-specific binding sites [14]. The same thing was used in 2009, has been developed a piezoelectric biosensor using HAp/glutaraldehyde/glucose oxidase to detect glucose [47].

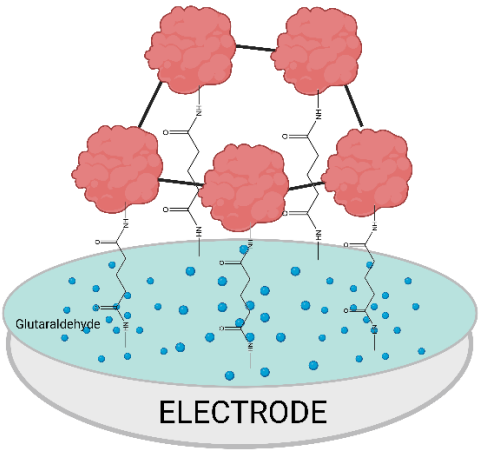

Figure 4. Illustration of cross-linking immobilization technique between biomolecules on the surface of the hydroxyapatite matrix and its composites using glutaraldehyde.

4.3. Covalent bonding.

This technique is very stable so that biomolecules with specific orientations will be strongly bound to the matrix on the electrode surface [48]. Generally involves the covalent bond between the matrix and protein amino acid chains, such as lysine (amino group), cysteine (thiol group), aspartic acid and glutamic acid (carboxylic group), hydroxyl group, imidazole group, phenol group, and so on. These groups are nucleophiles and tend to bind to electrophilic groups in the matrix to bind biomolecules to the matrix. It is often necessary to activate the matrix by adding reactive groups such as aldehydes, carboxylates, epoxies, and N-hydroxysuccinate (NHS) [49]. The most popular and used modifiers, such as APTES, VTES, and CPTES, and activating agents, such as EDC/NHS applied to the grafting process. Moreover, functional groups of biomolecules play important roles in the immobilization by covalent bonding [50].

Usually, this covalent bond occurs with the formation of an amide bond [38]. In 2017, has been developed a sandwich immunosensor using HAp/chitosan/anti-AFP as a probe in the GO layer. The large specific surface area and good GO conductivity increase the intensity of the molybdophosphate redox current. The carboxylate group in GO is activated using EDC-NHS to give a reactive succinimide ester which will react spontaneously with primary amines

or other nucleophiles through covalent bonds, one of which is amine coupling, as shown in Figure 5 [51].

In addition, there was also covalent bonding in the development of aptasensors for detecting MCF7 cells (breast cancer) using hydroxyapatite composited with ionic liquid 1-ethyl-3-methylimidazolium alanine and gold nanoparticles. Immobilization of thiolated aptamers to AuNPs by self-assembly via covalent Au-S bonds and ionic liquid 1-ethyl-3-methylimidazolium alanine with high ionic conductivity was placed on the electrode surface [52].

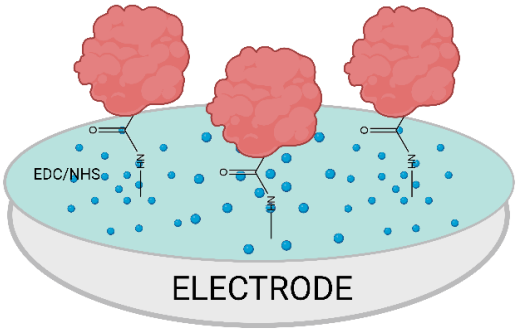

Figure 5. Illustration of the immobilization technique of covalent bonds between biomolecules on the surface of the hydroxyapatite matrix and its composites.

4.4. Non-covalent bonding with electrostatic interaction.

This immobilization technique is simple but less stable because it takes advantage of the ability to control the charge of the exposed groups for immobilizing biomolecules [38]. In 2007, has been developed a piezoelectric biosensor using HAp/GNP, HAp with an isoelectric point (IEP) at pH of 7.3 with a weak positive charge can be assembled with electronegative GNPs at pH of 7.0 through electrostatic interactions. Calcium ion (Ca^{2+}) is the positive surface side that can bind negatively charged molecules [11], and the phosphate ion (PO_4^{3-}) has a negative charge, and amino acid side chains on the protein are positively charged, which results in binding specific [53].

In the same method, in 2021, has been developed a DNA biosensor to detect Mycobacterium tuberculosis (M.tb) using multi-walled carbon nanotubes (MWCNTs), polypyrrole (PPy), and hydroxyapatite nanoparticles (HAp-NPs). There is an electrostatic bond between the DNA phosphate group with a negative charge and the calcium group of HAp-NPs with a positive surface charge [54]. DNA-based biosensors have been used for many applications given their strong detection and functionality within diverse environments and have shown robust accuracy and high sensitivity [55].

Biomolecules and hydroxyapatite matrices generally interact through non-covalent interactions or physical adsorption, such as hydrogen bonding, van der Waals interactions, hydrophobic interactions, and ionic bonds. However, they are dominated by non-covalent electrostatic interactions [56]. The immobilization of molecules containing carboxyl groups on the surface of HAp was found to increase the adsorption of biomolecules. In particular, van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions are the important interactions involved in the adsorption of biomolecules to the surface [57].

5. Hydroxyapatite Application in Biosensor

Hydroxyapatite has an important role in biomedical applications, especially biosensor precipitation, because of its properties such as good ion exchangeability, high porosity, multi-adsorbing sites, and excellent affinity for biomolecules so that it has potential as a base material for biosensors. Hydroxyapatite is used as a composite to increase electron conductivity so that it can increase the current response and immobilization on the electrode surface. Hydroxyapatite in biosensor applications such as electrochemistry, piezoelectric, and optics has been widely used in the last two decades, as listed in Table 1.

5.1. Electrochemical biosensor.

Electrochemical biosensors have attracted attention and are widely used to detect several biomolecules, such as viruses, because they have been shown to possess several advantages, such as high sensitivity, specificity, and selectivity, as well as cost-effectiveness, fast response, ease of use, commercially portable, and inexpensive instrument [58]. In electrochemistry, the observed reaction will produce a measure of the current (amperometric), measure the potential or charge accumulation (potentiometric), measure the conductivity (conductometric) between electrodes in a measurable manner, measure the impedance or resistance (impedimetric), and measure the between current and voltage (voltammetric) [59]. Nanomaterials with unique characteristics have been widely applied for signal amplification to increase the sensitivity of electrochemical methods [60].

The process of detecting electrochemical biosensors can be carried out with a label in the form of a redox probe as a marker, as described by Huang *et al*. [51], and without a label where the direct immobilization of biomolecules as bioreceptors on the sensor surface as described by Lu *et al*. developed a tyrosinase biosensor in detecting phenolic compounds using HAp-NP/chitosan/AuNP/tyrosine which shows higher peak currents compared to AuNP/chitosan/tyrosinase and HAp-NP/chitosan sensors. The limit of detection of this biosensor is down to 5 nM [61].

In the same method, has been developed an electrochemical aptasensor using hydroxyapatite nanoparticles (HAp-NP) composited with jelly-fish collagen (JF collagen) to detect thrombin. SPCE modified using HAp-NP/JF collagen composite with drop casting showed high current peaks. The results showed a decrease in current proportional to the increase in bioreceptor attachment and the detected target analyte, limit of detection down to 0.25 nM as shown in Figure 6. Thrombin binding aptamer (TBA) was immobilized on the surface of SPCE by cross-linking technique between the amine group on the composite and the amine group on TBA with glutaraldehyde (aldehyde group) as a crosslinker [3]. Aptamers are relatively small compared to antibodies, which can only bind to large molecules. The use of aptamers in aptasensors has been of great concern to researchers due to their synthetic portability and functional design. Aptamers' functionalization with nanomaterials can change their conformation, which will interfere with the aptamers' binding so that the aptamers' binding is carried out with unmodified nanomaterials or with the addition of a linker [60].

A sandwich-type electrochemical immunosensor to detect AFP using HAp as a probe to assist measurements coated with chitosan to introduce amino groups for covalent Ab2 conjugation was developed in 2017. On the other hand, GCE was modified using GO by drop casting, a large specific surface area and good GO conductivity increased the intensity of the molybdophosphate redox current, then the carboxylate group on GO was activated using EDC-

NHS and capture antibody or anti-AFP was immobilized on the surface. Incubated with BSA to block non-specific binding to the electrode surface and immobilized the HAp nanoprobe, the results showed a significantly higher current peak, indicating the capture of the nanoprobe to the electrode surface via antibody-antigen binding. This causes a decrease in an electrochemical current proportional to the detected AFP concentration. Detection with this immunosensor resulted in a detection limit of 50 fg/mL [51]. Immunosensor is a biosensor that involves the interaction of antigen and antibody where the antibody is an affinity biorecognition element [36].

In addition to immunosensors and aptasensors, electrochemical DNA-based biosensors have offered new opportunities since 2017 in the analytical field due to their properties in increasing DNA-based molecular diagnostic applications and taking full advantage of the existing modified electrode technologies [62].

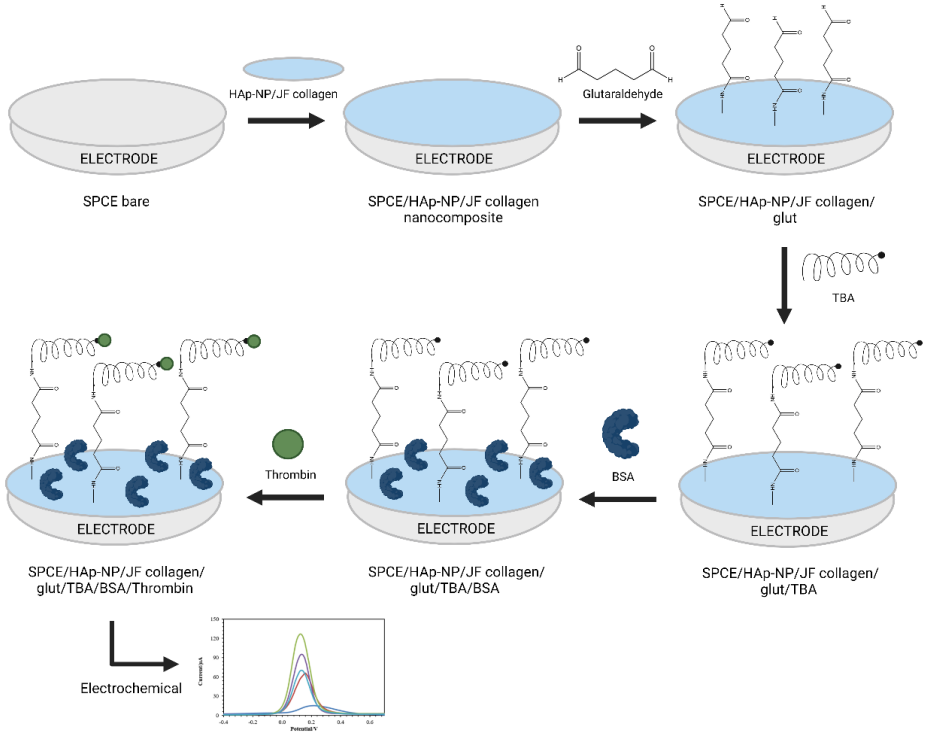

Figure 6. Schematic of electrochemical aptasensor to detect thrombin using hydroxyapatite nanoparticles/jelly-fish collagen. Adapted with modification from ref [3] and created by Biorender.

5.2. Piezoelectric biosensor.

In principle, the piezoelectric material works as an oscillator based on the piezoelectric effect, and the interaction with its surface is easily detected, so it is very suitable for the construction of biosensors that recognize affinity interactions. As a platform for the construction of the biosensor, quartz crystal microbalances (QCM) are used with a basic mode frequency of 1 – 20 MHz. Generally, a QCM sensor with a base mode frequency of 10 MHz and the electrode surface is gold-plated on both opposite sides [63]. Piezoelectric materials have also become important biomaterials that can be linked to biological tissues and used in bioelectronic devices [64].

In 2008, has been developed a piezoelectric immunosensor using a gold-coated 9 MHz QCM to detect α-fetoprotein (AFP). The result of the detection limit of this biosensor is down to 15.3 ng/ml, as shown in Figure 7. Electrode modification with GNP/HAp-NP using thiol self-assembly using cysteamine to produce an amine group on the surface so it can bind to

GNP/HAp-NP. Anti-AFP immobilization on the surface of HAp-NP/GNP with electrostatic interactions due to the multi-adsorbing sites of HAp [53].

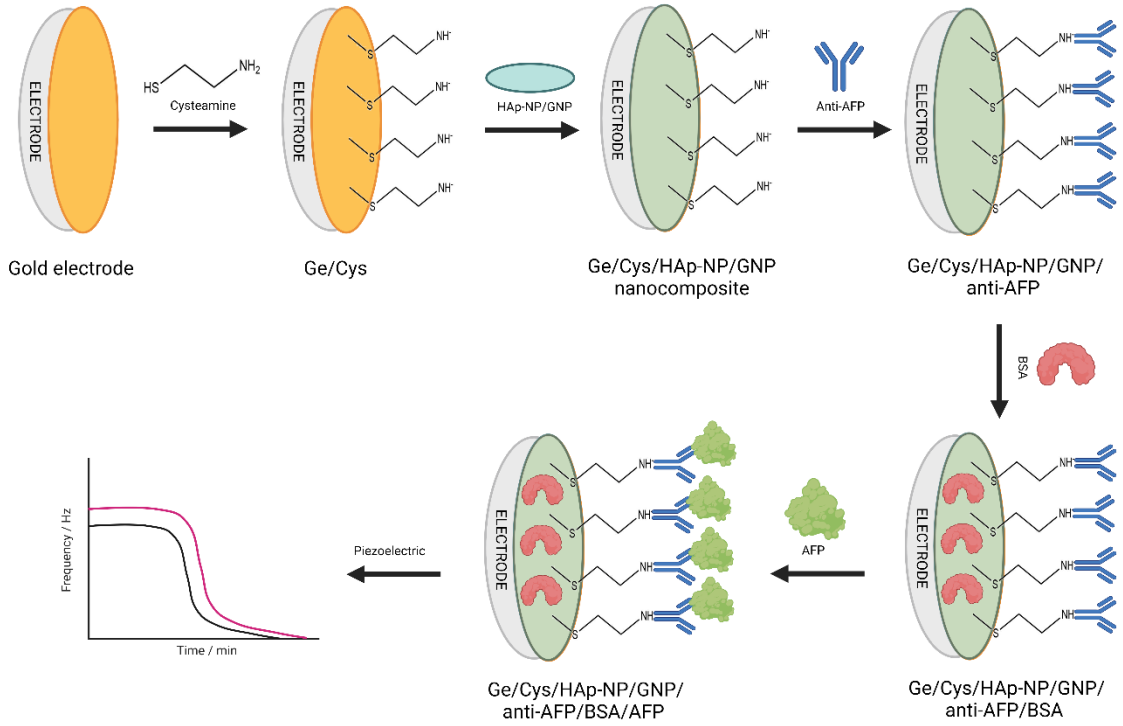

Figure 7. Schematic of piezoelectric immunosensor to detect α-fetoprotein using hydroxyapatite/gold nanoparticles. Adapted with modification from ref [53] and created by Biorender.

5.3. Optical biosensor.

Optical detection that utilizes the interaction of optical fields or measurement of light emitted or absorbed resulting from the biochemical reaction between analyte and bioreceptors is a class of biosensors reported quite frequently [31]. The detection process can be done with a label and without a label. Label-free sensor, the detected signal is generated directly by the interaction of the target analyte with the transducer. In contrast, labeled sensors involve the use of a probe and an optical signal generated by colorimetric, fluorescence, surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), or luminescence methods [65,66]. Fluorescence probes and SPR are found widely in various bioassays and bioimaging applications due to their high throughput, high sensitivity, and ease of use in vivo by utilizing electromagnetic waves to detect changes in the immobilization reaction of bioreceptors and analytes [31].

Table 1. The use of hydroxyapatite in biosensor applications over the last two decades.

NO	ELECTRODE	BIORESEPTOR	TARGET	LOD	REFERENCES
			Electrochemical		
1	GCE	HAp-NP/PPY/ MWCNTs/DNA probe with methylene blue label	Mycobacterium tuberculosis (M.tb)	0.141 nM	[54]
2	GCE	HAp	Cysteine (CySH)	0.03 µmol/L	[15]
3	GCE	HApNP/ssDNA/Metilen Blue (MB)	BK polymavirus	41.08×10^{-12} mol/L	[68]
4	GCE	Fe-HAp-Ab2 modified GCE/TP-COOH NCs/ProtA/Ab1	Cytokeratin 19 fragment 21−1 (CYFRA21−1)	0.01471 pg/mL	[69]

NO	ELECTRODE	BIORESEPTOR	TARGET	LOD	REFERENCES
5	GCE	HAp/rGO/GNP/Uricase (UAO)	Uric acid	3.9 x 10^{-6} M	[26]
6	GCE	HAp-TBA2 modified GCE/MNP-TBA1/SP	Thrombine	0.40 fM	[70]
7	GCE	HAp-TBA2 modified GCE/MNP-TBA1	Thrombine	0.03 fM	[71]
8	GCE	HAp/Chitosan/Ab2 modified GCE/GO/Ab1	α-fetoprotein (AFP)	50 fg/mL	[51]
9	GCE	HAp/Aptamer/anti-PDGF-BB modified GCE/GO	Cancer biomarker platelet-derived growth factor BB (PDGF-BB)	50 fg/mL	[72]
10	GCE	Ce-HAp	Norepinefrin (NE), uric acid (UA), and tyrosine (Tyr)	0.058 µM, 0.39 µM, dan 0.072 µM	[73]
11	GCE	Graphene nanosheets/HAp	Matrine (MT)	1.2 µM	[74]
12	GCE	HAp-rGO-Chitosan	Hydrazine	0.43 µM	[75]
13	GCE	Fe-HAp/MWCNT	L-dopa	0.062 µM	[13]
14	GCE	GO/HAp/Ab	α-fetoprotein (AFP)	5 pg/mL	[76]
15	GCE	HAp/rGO/glucose oxidase	Glucose	0.03 mM	[77]
16	GCE	Magnetik Fe_3O_4/HAp/MIPPy	Bilirubin	0.007 µM	[78]
17	GCE	Fe-HAp/tirosinase	L-tirosin	245 nM	[79]
18	GCE	HAp	Folic acid	75 nM	[80]
19	GCE	Meso-HAp/TiO2/MWCNT	Glucose oxidase (GOx)	2 µM	[81]
20	GCE	Prussian blue@HAp-HRP-Ab2 modified GCE/Thionine/Graphene Sheet/Ab1	α-fetoprotein (AFP)	9 pg/mL	[14]
21	GCE	HAp nanowires/chitosan/HRP	Cyanide	0.6 ng/mL	[82]
22	GCE	HAp-MWCNT/Hb	Hydrogen peroxidase (H_2O_2)	0.09 µM	[83]
23	GCE	HAp/GNP-Hb	Hydrogen peroxidase (H_2O_2)	0.2 µM	[44]
24	GCE	silica/HRP–HAp	Hydrogen peroxidase (H_2O_2)	0.35 µM	[84]
25	GCE	HAp/Nafion/glucose oxidase	Glucose	0.02 mM	[85]
26	GCE	HAp/PDDA/Hb	O_2, H_2O_2, and NO_2-		[86]
27	GCE	HAp/L-Lysine	Nile blue A (NBA)	5.07 × 10^{-8} mol/L	[87]
28	SPCE	JF collagen/HApNP/TBA/ Aptamer	Thrombine	0.25 nM	[3]
29	SPCE	Ag-HAp-Nb19-Ab2 modified SPCE/Au/Nb11/Apo-A1	Apolipoprotein-A1	0.02 pg/mL	[88]
30	SPCE	PB-HAp-HRP	Hydrogen peroxidase (H_2O_2)	7 µmol/L	[89]
31	GE	IL/HAp nanorod-AuNP/ Aptamer/c-DNA tagged AgNP	MCF7 cells (breast cancer)	8 ± 2 cells/mL	[52]
32	GE	HAp-GNP-Chitosan/Anti-PSA	Prostate specific antigen (PSA)	2.6 ng/mL	[25]
33	GE	HApNP-chitosan-tyrosinase	Phenolic compounds	5 nM	[61]
34	GE	MPA/HAp/Divinylsulphone/ Anti-human transferrin	Transferrin	0.15 ng/mL	[90]

NO	ELECTRODE	BIORESEPTOR	TARGET	LOD	REFERENCES
35	PGE	HAp/double stranded DNA (dsDNA)	Phosphate labelled Hepatitis B virus (HBV) DNA probe	12.80 µg/mL	[91]
36	PGE	HAp/IL	Curcumine-DNA	1.86 µg/mL (equals to 5.04 µM)	[92]
37	CILE	Nafion/Mb-HAp@CNF	Myoglobin (Mb)	2.0 mM	[93]
38	CPE	Bio-waste HAp/rGO	Dopamine, acetaminophen, and uric acid	0.39, 1.32, dan 0.63 µM	[94]
39	CPE	γ-Fe_2O_3/HAp/Cu(II)	Metformine in urine	14 nM	[95]
			Pizoelectric		
40	quartz crystal microbalanc (QCM, 9 MHz, gold electrodes)	HAp/glutaraldehyde/glucose oxidase	Glucose	0.34 µmol/L	[47]
41	quartz crystal microbalance (QCM)	poli-L-lisin/HAp/CNT/ anti-CA19-9	Carbohydrate antigen 19-9	8.3 U/ml	[96]
42	quartz crystal microbalance (QCM, 9 MHz, gold electrodes)	HAp/γ-Fe_2O_3/Au/ anti-hIgG	hIgG	~15 ng/ml	[97]
43	quartz crystal microbalance (QCM, 9 MHz, gold electrodes)	Cysteamine/HAp/GNP/AFP antibody	α-fetoprotein (AFP)	15.3 ng/ml	[53]
			Optical		
44	Fluorometric	HApNP	Cysteine and homocysteine	110 nM and 160 nM	[67]
45	Fluorometric	HApNP	Protein kinase A (PKA)	0.5 U/L	[98]
46	Fluorometric	HApNP/TPEHP-CB[6]	Spermine and Spermidine in urine and blood	1.4×10^{-8} and 3.6×10^{-8} M	[99]

In 2018, the fluorescence of HAp-NP by doping with Eu3+ and utilizing HAp-NP for fluorometric detection of biothiol was synthesized. The synthesized HAp-NP is rod-shaped, has two strong fluorescence emission peaks, and has good water solubility, making HAp-NP a good candidate for a fluorescence probe. Cu^{2+} can quench the fluorescence of HAp-NP, while the addition of biothiols such as cysteine (Cys) and homocysteine (hCys) can cause fluorescence recovery due to the strong bond between Cys and hCys with Cu^{2+}. This detection resulted in a detection limit of 110 nM for Cys and 160 nM for hCys [67].

6. Conclusions

Hydroxyapatite and its composites have played an important role in biosensor applications with various electrode surface immobilization techniques over the last two decades. Hydroxyapatite and its composites are used as matrix components for bioreceptor immobilization because of their large surface area, stability, multi-absorbent sites, and affinity for biomolecules. Gold nanoparticles show good composites for hydroxyapatite due to their high electron conductivity with a small limit detection value of 2.6 ng/mL. Adsorption techniques demonstrated good immobilization techniques for bioreceptor on hydroxyapatite and its composites in the presence of hydrogen bonds, Van Der Waal forces, hydrophobic interactions, and electrostatic interactions.

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Conflicts of Interest

The authors declare no conflict of interest.

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