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A review on electrochemical biosensing platform based on layered double hydroxides for small molecule biomarkers determination

Muhammad Asif,[1'2] Ayesha Aziz,[1] Muhammad Azeem[1], Zhengyun Wang[1], Ghazala Ashraf[1], Fei Xiao[1]*, Xuedong Chen[2]*, Hongfang Liu[1]*

1 Key Laboratory for Large-Format Battery Materials and System, Ministry of Education, Hubei Key Laboratory of Material Chemistry and Service Failure, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan, 430074, P. R. China.

2 State Key Laboratory of Digital Manufacturing Equipment and Technology, Huazhong University of Science and Technology, Wuhan 430074, P. R. China

Abstract

The development of layered double hydroxides (LDHs), also known as anionic clays with uniform distribution of metal ions and facile exchangeability of intercalated anions, are now appealing an immense deal of attention in synthesis of multifunctional materials. In electrochemical biosensors, LDHs provide stable environment for immobilization of enzymes or other sensing materials and play crucial roles in development of clinical chemistry, point-of-care devices through analysis of various small molecule metabolites excreted by biological processes which in turn serve as molecular biomarkers for medical diagnostics. In this review, we summarize the recent development in fabrication of LDH based nanoarchitectures and their electrocatalytic applications in ultrasensitive *in vitro* determination of conventional biomarkers, *i.e.*, H_2O_2, glucose, dopamine and other biomolecules. Moreover, detailed discussion has been compiled to differentiate electrochemical enzymatic and nonenzymatic biosensors, to evaluate useful concentration ranges of H_2O_2 and glucose for analytical circumstances and to distinguish tumorigenic and normal cells *via* quantifying the released H_2O_2 efflux from living cells. Here, we envision that electrochemical sensing platform based on structurally integrated LDH nanohybrids with highly conducting substrates will assist as diseases diagnostic probe further enhancing diagnosis as well as therapeutic window for chronic diseases. Finally, the perspective for fabrication and assembly of LDH electrode is proposed for the future innovation of

electrochemical biosensors with high performance making them more reliable for *in vitro* diagnostics.

Keywords: Layered double hydroxides; Modified electrodes; Electrochemical biosensor; Biomarker determination; *In vitro* diagnostics

Contents

1. Introduction

With the ongoing gigantic investigation of nanoscale materials, layered double hydroxides (LDHs) also known as hydrotalcite-like compounds have emerged as rapidly increasing research themes. As presented by Fig. 1A, LDH is a class of two dimensional (2D) anionic lamellar clay materials of alternately packed brucite like positively charged layers with charge compensating anions and solvation molecules present between the interlayer regions. Unlike silicate based cationic clays, majority of which present naturally on the Earth, only a few anionic clays have been found in nature while most of them are prepared artificially in the laboratories. The metal cationic species present insides the edge sharing octahedral along with their vertexes containing hydroxide ions that interconnect to create ultimately 2D sheets of LDHs [1, 2]. As a mineral having intriguing properties, it was first discovered and synthesized in laboratory facilities in Sweden as well as registered as a first patent of hydrotalcite-like compounds in 1970 by Brocker [3]. The chemical composition of extensively studied LDHs are symbolized by generic formula of $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2][A^{n-}]_{x/n}\cdot zH_2O$, where M^{2+} represents commonly used divalent species (like Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ru^{2+}, Rh^{2+}, and Zn^{2+}) and M^{3+} expresses some commonly used trivalent species (such as Al^{3+}, Mn^{3+}, Fe^{3+}, Co^{3+}, Ni^{3+}, Cr^{3+}, Ga^{3+}). Here A^{n-} depicts the nonframework charge balancing anions or hydroxides (*e.g.* CO_3^{2-}, Cl^-, SO_4^{2-}, RCO_2^-, OH^-) located in the interlamellar region in order to restore the charge neutrality and value of *x* always remains in between 0.2-0.4 [4, 5]. Some other anions have also been inserted in lamellar structure including carboxylate, dicarboxylate, alkanosulfonate, $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$, polyoxometalates and now graphene nanosheets etc. [6].

The distinctive assets concerned with LDHs is the interchange of divalent cations like M^{2+} by some trivalent cations like M^{3+}, which are responsible for the creation of positive holes within the brucite type layers and this positive charge is balanced by the presence of exchangeable inorganic/organic anions in the interlayer galleries, consequently LDHs serve as p-type semiconductive channels [7, 8]. Moreover, anion exchangeability of LDH offers the intercalation of chelating agents within the layers. For instance, intercalation of ethylenediaminetetraacetic acid (EDTA) and thiol based chelating reagents increases the performances of targeted sensing abilities. The homogeneous mixed oxides produced after thermal decomposition of LDHs precursors have captured immense attention because of the existence of M^{2+} and O^{2-} as acid-base pairs, which are key factors imparting particular catalytic properties. Moreover, metal cations of

LDHs become more disperse and create highly homogeneous mixture of metal oxides after thermal decomposition that ensure improved catalytic activity.

Fig. 1. (a) Ideal structure of Mg^{2+} and Al^{3+} layered double hydroxide with carbonate anions in interlayer gallery, (b) SEM image of MgAl LDHs, (c) Schematic illustration of LDHs nanomaterials employed as biosensing materials for electrochemical determination of biological analytes.

Over the last two decades, properties of LDH materials have been tremendously explored to fabricate new scaffold of materials for their widespread practical applications in numerous fields. LDHs being low cost and nontoxic for bio-organisms have sparked a wealth of interest because of their application as electrocatalysis, efficient therapeutic capabilities in the treatment of various diseases and more significantly in electrochemical biosensing platform as biomarker determination for early diseases diagnosis. Electrochemical biosensors offer robust and precise measurements with economical and simple instrumentations and are capable of using new nanomaterial based technologies to enhance the performance of biosensors for biomarker determination [9]. *In vitro* diagnostics need certain biomarkers for early disease diagnosis [10, 11]. In biotic fluids, small metabolic molecules *e.g.*, glucose, hydrogen peroxide (H_2O_2) and dopamine with specific concentrations indicating definite pathological processes might work as biomarkers to be used for early diseases diagnosis [12, 13]. For instance, disorder management in blood glucose level is recognized as an important index in several chronic metabolic diseases such as diabetes. The diagnosis of diabetes can be carried out in estimating plasma glucose concentration which should be ≥7.0 mM (or ≥11.1 mM 2 h after orally loading 75g glucose). On the other hand, natural metabolic byproduct of oxygen such as H_2O_2 plays critical role in normal cell signaling, steady development and homeostasis [14, 15]. Recent studies have exposed that irregular H_2O_2 level is considered to be associated with many incurable diseases such as lungs injury, cardiovascular diseases, parasitic infections and tumor development [16]. Therefore, *in vitro* determination of H_2O_2 is of great importance as emerging recognize biomarker for screening, monitoring and diagnostics of cancer and many other associated disorders. Dopamine (DA) is one of the widely studied monoaminergic neuroreceptors which is considered as signaling molecule for precise determination of various kinds of neural diseases. It is well known that inflexible psychiatric and fatal neurodegenerative disasters such as Huntington's, Alzheimer's, and Parkinson's diseases are strongly related with DA fluxes. There is always high

probability for dopaminergic neurons to be destroyed before it is clinically diagnosed [17]. Hence it is extremely mandatory to monitor the exact levels of biological molecules to better understand the proper functioning of biomarkers for more accurate development of early diseases diagnostic tools.

In this review, we outline the recent development in the fabrication of LDH based nanoarchitectures and explore their practical applications in ultrasensitive *in vitro* determination of small biological molecules of interest. The functions of several LDHs in electrochemical sensors and the performance of these sensors in *in vitro* detection of emerging biomarker i.e., H_2O_2, glucose and dopamine have been discussed in detail. Benefitting from ultrasensitivity, good selectivity and fast response time, electrochemical sensing platform can also be used in *in vitro* determination of biotic molecules from human samples and real-time monitoring of these biomarkers from live cells. Furthermore, we believe that this nano-biosensing platform based on structurally integrated LDHs materials will enhance early diagnostic as well as therapeutic window of diseases.

2. Unique characteristics of LDHs nanostructures

LDH nanostructures are attractive candidates for the modification of electrodes because of the following unique characteristics [18, 19].

- LDHs can easily be prepared through different synthesis methods.
- The precursors used for LDHs fabrications are of economical.
- LDHs ensure improved mobility to analytes and other reaction products because of porous nature.
- There are always strong interactions of LDHs with polar as well as negatively charged molecules owing to high positive charge on layers.
- This excessive positive charge on LDH layers is neutralized by replaceable anions in layers.
- LDHs offer good biocompatibility for entrapping molecules in virtue to high water content.
- LDHs afford a surface where enzymes can maintain their activity for long period of time.
- Excellent hydrolytic and chemical stabilities are associated with LDH materials.
- LDHs facilitate facile loading of catalysts.
- LDHs play vital role in enhancing sensitivity and selectivity of electrochemical biosensors.

- LDH nanostructures, when used in electrochemical sensing systems, serve as one or more of the following parts.
  - Electrocatalysts
  - Adsorbents for stripping analysis
  - As surface to immobilize some biomolecules or other modifiers

3. Synthesis of LDHs

3.1. Simple coprecipitation method

Typically, in co-precipitation method, gradual addition of a salt solution comprising of target anions which are to be intercalated into a vessel having mixed solution of divalent and trivalent cations of metallic salts in an appropriate ratio. Finally the pH of solution is increased by adding a base or urea hydrolysis that ensures the swift precipitation of LDH. These interlayer anions counter positive layers of LDH. To increase the crystallinity of the sample, thermal treatment or aging of mixture is commonly performed after co-precipitation process. The pH of mixed metal salts is maintained at a desired level by adding alkali which leads the co-precipitation of the two metallic salts. The mechanism of co-precipitation is preferably explained on the basis of condensation of hexa-aqua metal complexes in solution, leading to the formation of brucite-like sheets with uniformly dispersed metal cations and solvated interlayer anions. Most commonly, LDH materials are synthesized by traditional coprecipitation method in a basic condition under gradual mixing of precursor metallic salts. Briefly, divalent and trivalent metal ions holding the radii probably close to Mg^{2+} constitute the host layers of LDH nanostructures [20]. It is more simple and cost-effectiveness method and is mostly employed for LDH synthesis to be used in electrochemical sensor development. Following essential components are required to prepare LDH by coprecipitation method.

- Divalent and trivalent soluble cations would be needed to form layers.
- Charge balancing interlayer anionic species which counter positively charged layers of LDH.
- To start LDH precipitations, a strong base is required.

Majority of the electrochemical sensors have been constructed by LDH materials fabricated by coprecipitation method. For example, Liu's group [8] fabricated MnO_2 nanoparticles intercalated in the host layers of MgAl LDH and explored its practical applications in real-time determination of H_2O_2 from live cancer cells (Fig. 2.). Due to synergistic effect of

semiconductive LDH and highly loaded MnO_2 nanoparticles, the synthesized MnO_2MgAl LDH nanohybrid material showed superior electron transport, enriched surface active sites and improved catalytic activity. LDHs are prepared by this strategy allowing several enzymes to be immobilized keeping their reactivity unaffected. The enzyme based biohybrid LDHs like urease, trypsin, [21] and alkaline phosphatase [22] have been synthesized. Under controlled conditions, the quantity of enzyme immobilized and interaction with LDH layers can be well-precised by adjusting the charge density of host layers along with enzyme/LDH ratio. Because of inherent characteristics of protein such as surface charge density, hydrophilic domain and its ability to be repartited, remarkable variation in immobilization rate has been witnessed. Nevertheless, transition metal based LDHs are challenging to fabricate by homogeneous coprecipitation due to the fact that few transition metals specifically Fe^{3+} possess the tendency to engender gel-like hydroxides at about low pH values [23]. Moreover, LDHs with core-shell architecture and hierarchical sphere like morphology can also be synthesized by coprecipitation approach. In this process, when a substrate absorbs definite amount of cations from mixture of metallic salt solution, precipitation will arise on substrate surface, which subsequently results in LDH crystal growth through interface nucleation. The adsorbed LDH crystallites on the surface substrate are also facilitated by the particle-particle association of LDH nanoparticles in the solution.

Fig. 2. Schematic illustration of fabricated MnO_2 MgAl LDH hybrid material and SEM images at different magnifications [8].

Recently, Zhan and Liu's groups [24, 25] synthesized hybrid nanospheres of LDHs and studied their electrocatalytic potential in oxygen reduction reactions as well as electrochemical biosensor for H_2O_2 detection. The results revealed the excellent performance of NiFe-LDH/NrGO nanospheres in three aspects such as N doping, porous surface, strong coupling affinity between LDH and rGO endowing abundant surface active sites and suitable mass-transfer aptitude. The superb presentation of CuO@MnAl LDH nanospheres in H_2O_2 detecting platform has been observed due to enhanced charge transfer abilities at breakdown voltage of p-n junction formed by the intercalation of n-type CuO spinels with p-type semiconductive channels of MnAl LDH. In addition, MnAl LDHs with abundant positive holes and marvelous intercalation mechanics enhanced considerable percentage mass loading of CuO NPs in their

host layers, which granted the nanospheres with numerous surface active sites to adsorb much more H_2O_2 molecules. The core-shell morphology can be achieved by modifying the surface of magnetic particles with negatively charged functionalities to be employed as core substrate for the growth of LDH shells. For example, carbon-coated Fe_3O_4 nanospheres with numerous hydroxyl groups on the surface was efficiently used as core when uniform suspension was obtained by ultrasonication in NaOH solution and methanol then another mixed solution of $Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$ in methanol was poured dropwise in vigorously stirring above suspension with controlled pH of 10 and subsequently treated hydrothermally to carry out the resultant core-shell Fe_3O_4@C@LDH nanostructures [26, 27]. The hydrothermal treatment time and temperature can be responsible to control the particle size and thickness of LDH shell. Magnetic nanoparticles (MNP)-core and LDH-shell hybrid nanospheres were synthesized by adding magnesium ferrite particles in a mixed solution of Mg^{2+}, Al^{3+} ionic salts and diclofenac (DIC) as drug molecule [28].

Fig. 3. Schematic protocols to synthesize magnetic-core/LDH-shell composite nanosphere [14].

The DIC has been intercalated in between the interlayer regions and then coated on the surface of core together with LDH phase. Apart from the methodologies in which the surface of substrate needs to be modified by means of chemical processes, the negatively charged surface of core can also be established by tuning the pH values as well (Fig. 3.). The hierarchical LDH shell was fabricated through interface nucleation and crystal growth progressions by dropwise addition of metallic salt solution in a constantly stirring suspension of Fe_3O_4 with controlled pH of 10 [29]. It is noteworthy that the surface of as-prepared Fe_3O_4@MgAl LDH nanospheres can further be modified to produce additional functionalized nanostructures. In this work, Au nanoparticles supported Fe_3O_4@MgAl LDH nanospheres has been fabricated by deposition-precipitation method [30]. The combination of LDH nanoplatelets with magnetic particles by coprecipitation method is a facile and effective approach to construct core-shell nanoarchitectures.

3.2. Layer-by-layer assembly

The phenomenon of spontaneous and uninstructed structural reconstruction which consequently creates disordered system is known as self-assembly. The layer-by-layer (LBL)

self-assembly has arisen versatile and easy way to develop ultrathin multilayers because of well-defined structures as well as controlled chemical composition. LDH materials have been shown to be LBL self-assembled. The self-assembly of LDH nanosheets with other functionalities can be established by depositing exfoliated LDH nanosheets on the surface of substrate as primary building blocks. Li et al. [31] used LBL self-assembly approach to construct core-shell structure with polymer as core and delaminated LDH nanosheets as shell. The core-shell nanospheres were synthesized by coating exfoliated nanosheets of LDH on the surface of magnetic core like Fe_3O_4 crystalline particles [32]. The layers of silica were deposited on the surface of magnetic particles by sol-gel process. Subsequently, the as-prepared $Fe_3O_4@SiO_2$ spheres were homogeneously mixed first in formamide suspension of exfoliated LDH nanosheets and then in a basic solution of Na_2CO_3 repeatedly. The as-obtained product was heated at 480˚C for 4 h, and then dispersed in aqueous solution to restore original platelet like structure of LDHs. The monodispersed spherical structure of Fe_3O_4-core and silica-shell was retained after deposition of 20 layers of LDHs. The LBL self-assembled core-shell nanohybrids inherently contain the intrinsic assets of both of magnetic spheres and LDH crystallites, which are liable to build advanced multifunctional hybrid materials. The driving forces in LBL self-assembly process like hydrophobic association and hydrogen bonding is the beauty of this methodology that widens its feasibility in fabricating advanced multifunctional core-shell magnetic particles-LDH nanocomposites compared with only electrostatic forces involved in reassembly process which confines the further applicability of these nanohybrid materials.

Moreover, hierarchical LDHs core-shell nanostructures with multifunctionalized micro- and nano-species imparting intriguing properties has been recently fabricated using sol-gel process followed by in situ growth as in Fig. 4 [33]. Firstly, the surface of Fe_3O_4 nanosphere was coated with thin layer of silica by means of sol-gel process to enrich the surface with negative functionalities, and secondly several layers of AlOOH primer were coated on the surface of $Fe_3O_4@SiO_2$ by LBL strategy. The last step is to generate LDH nanoplatelets on the surface of $Fe_3O_4@SiO_2@AlOOH$ by the process of in situ growth tactics to fabricate $Fe_3O_4@SiO_2@LDH$ core-shell nanospheres.

Fig. 4. Schematic illustration of fabrication of Fe_3O_4@SiO_2@LDH nanospheres, TEM images of A) Fe_3O_4 particles, B) Fe_3O_4@SiO_2, C) Fe_3O_4@SiO_2@AlOOH, D) Fe_3O_4@SiO_2@NiAl-LDH microspheres demonstrating the Fe_3O_4-core/NiAl-LDH shell structure [33].

It is noteworthy that without AlOOH coating, the core-shell spherical morphology of Fe_3O_4@SiO_2@LDH cannot be achieved, which authenticates that AlOOH coating is the key feature to afford the required sources of aluminum for nucleation and growth of LDH nanoplatelets. Moreover, the coating of SiO_2 offers the essential interactions to firmly immobilize AlOOH on the surface by hydrogen bonding. Different morphologies of LDH microspheres with tunable architectures like core-shell, yolk-shell, and hollow inside assemblies were also fabricated by in situ growth process [34, 35]. In recent studies, the LDH nanoplatelets with high crystallinity have also been fabricated hydrothermally by employing urea and hexamethylenetetramine as hydrolysis reagents [36]. In this process, the progressive hydrolysis agents like urea or hexamethylenetetramine make the solution alkaline and encourage homogeneous nucleation process as well as the crystallization of LDH product. In addition to hydrothermal process, there is another route to create crystalline hexagonal LDH material by topo-chemical transformation through which researchers synthesized series of LDH micro platelet structures [20]. The imperative feature of this strategy is the rational control of oxidation states of metals in hydroxide precursor solution [37].

3.3. Single layer and superlattice LDHs nanosheets

The ever increasing application potential of LDH materials in terms of catalysis, catalyst precursors, anion exchangers and electroactive as well as photoactive materials still have somewhat limitations due to the inaccessibility of inner species of interlayer region. The effective way to solve this conundrum would be the fabrication of single crystal anisotropic layer of LDH nanosheets, which consequently possess enlarged surface area with entirely exposed electrochemically active surface sites [37]. The top-down and bottom-up approaches are being used for synthesis of LDH nanosheets. The process of exfoliation or delamination of bulk LDHs to harvest monolayer nanosheets by top-down mode is carried out through exchange of intercalated anions with other larged size anionic surfactants to expand interlayer spacing which results in weakening the brucite type interlayer region attractive forces [38]. The most commonly

used solvents to carry out exfoliation processes are extremely polar in nature similar to butanol, [39] toluene and CCl_4, [40, 41] acrylates, [42] formamide, [6, 23] water [43, 44] and others also, these solvents actually associate with hydrophobic parts of interlamellar anionic species through the process of solvation [45]. Besides this method, exfoliated LDH nanosheets can also be synthesized by bottom-up strategy in which coprecipitation of aqueous precursor solution occurs in a water-in-oil reverse microemulsion system with surfactants. In this strategy LDH single layer is formed by inverse micelles microemulsion nanoreactors within the accessibility of confined nutrients in a limited space.

Fig. 5. (a) Schematic procedures to exfoliate bulk LDHs into single layer nanosheets and superlattice self-stacking heteroassembly of LDH and graphene nanosheets, (b) HRTEM and schematic illustration of alternately stacked LDH nanosheets and graphene superlattice[46].

A fewer number of such controlled nucleation examples MgAl LDH, [47] NiAl LDH [48] and CoAl LDH[49] monolayer nanosheets have been reported. Additionally, there are some limitations in both quantity and diversity for successful fabrication of LDH monolayer sheets by bottom-up approach because of stringent control on oil and water phase as well as concentration of metallic salt solutions. Therefore upto this end, top-down synthesis process for generating single layer LDH nanosheets is the most extensively applied method.

In particular, the applications of LDH based materials have been confined owing to the insulating nature of metal oxides/hydroxides [2]. To circumvent the bottleneck of these problems, molecular-level hybridization of extremely thin single layer nanosheets of LDH together with monolayer of highly conductive carbon substrates such as carbon nanotubes, conductive polymers or graphene may be an ideal strategy to accomplish superb electrocatalytic performances due to direct neighboring of conductive substrate and catalytic metal oxides/hydroxides. More recently, superlattice type composite nanomaterials assembled by inserting highly ultra-conductive single layers of polymer, carbon nanotubes and graphene within the interlayer gallery of LDH materials [6, 46] were reported as shown in Fig. 5. The inherent electrochemical capabilities, when further enhanced through the development of appropriate nanosheets architectures, will be critical in holding intriguing applications of LDH based materials in electrochemical biosensing platform, which will be under discussion to some extent in the following segment.

3.4. Electrodeposition approach

Electrodeposition is known as conventional surface modification technique which is emerging and versatile method to fabricate numerous nanomaterials with variety of different morphologies such as thin and thick coatings, free-standing sheets, foil, wires, plates and even powders as well. It is single step process which is operated at low temperature and produces fully dense nanostructures. Recently, facile and green approach like electrodeposition has been used to fabricate LDH based nanostructures impressively broadening the landscape of LDH fabrication strategies. To solve the issue of insulating character of LDH, CoNi LDH has been electrochemically deposited on conducting polymer such as polypyrole (PPy@CoNi LDH) [50] where PPy provided good conductivity and CoNi LDH served as host receptor for anions. Moreover, CoFe LDH and NiCo LDH wrapped CuO and ZnO nanowires [51, 52] based materials have been synthesized by different researchers with diverse morphologies employing electrodeposition method. The most interesting is the electrochemical synthesis of CdAl LDH and CoFe LDH directly on the polished surface of indium tin oxide (ITO) and glassy carbon electrode (GCE) to be used in energy storage devices [53, 54]. This approach is considered superior over others because of several advantages such as less toxic organic solvents are required during synthesis, time saving, easy to operate and mild condition for fabrication. Few researchers have used ultrasonic wave assisted methods to synthesize CoAl LDH [55], where the mixture of precursor salt solutions in appropriate proportion is firstly sonicated then heated at certain temperature and refluxed for some time before allowed to cool at room temperature. In addition to nanoparticles, nanoplatelets and core-shell assemblies, several research groups have also reported certain other structural architectures of LDH based materials with nanocone and nanoflower [56] like morphology. Forticaux et al. [56] studied the prototypes of ZnAl LDH and CoAl LDH with the conclusion of LDH growth driven by screw dislocation. Accordingly, well-defined distinct morphologies of LDH nanoplates have been developed with controlled and sustained low precursor supersaturation compared with uncontrolled overgrowth of LDH nanoplates that resulted in nanoflower architectures.

3.5. Comparative study among the employed approaches

Comparing the above four approaches of LDHs fabrication, the summaries are as below;

- Co-precipitation approach is simple, cost-effective, easy to immobilize enzymes and more suitable taking yield into consideration; in this case, yield of the product is three times more

compared with other three routes. While synthesizing core-shell structure, there is no need of surface modification by chemical process, just pH adjustment is enough. Additionally, several parameters such as temperature, pH of reaction medium, concentration of alkali solution and anion species, etc. can be controlled well during the preparation by this method.

- The authoritative feature of layer-by-layer assembly is the rational control of oxidation states of metals in hydroxide precursor solution. It can develop ultrathin multilayers because of well-defined structures and controlled chemical composition. The shell of desired thickness with the deposition of many layers can be generated on the modified surface of core. However, this method seems to be time consuming, lengthy and less efficient.
- Single layer and superlattice approach is used to create monolayers with enlarged surface area. The major issue related with LDHs is poor conductivity which can be improved by this superlattice strategy through atomic level hybridization with conductive substrates such as CNTs and graphene.
- Electrodeposition approach is a single step and versatile method to synthesize LDHs with variety of morphologies such as thin and thick coatings, free-standing sheets, foils, wires and even powders. This approach is considered superior over others because of several advantages such as less toxic organic solvents are required during synthesis, time saving, easy to operate and mild condition for fabrication.

4. LDHs based electrochemical biosensors for biomarker determination

The electrochemical biosensors have been advertised as a particularly promising class of imminent generation of analytical devices with fascinating characteristics because of incredible performance superior to conventional analytical tools since the successful demonstration of biosensor by Leland C. Clark. [57] Considerable attention has been devoted by numerous researchers to achieve improved functionality of this device for the determination of several small molecular metabolites. During past decade, electrochemical sensors and biosensors have conquered a wealth of interest because of facilitating multiplexed, simultaneous determination of various analytes at low concentration as well as exciting features like economical, simplicity, biocompatibility and excellent sensitivity. [58] With the ongoing gigantic demands of fast point-of-care medical strategies, biosensors are being extensively used in hereditary investigation, human immunodeficiency virus, angiogenesis, and metastasis [59]. The *in vitro* sensing of

biological molecules and cellular targets from blood serum, urine, honey, living systems and tumorigenic environments are considered beneficial over *in vivo* analysis [60] because of simplicity in environmental manipulation, minimum operational instrumentation, disposable, as well as portable benefits [61], which are critically important for prompt on-site diagnosis of diseases. More recently, electrochemical sensors are considered as extremely sensitive platform for detecting various chemical and biochemical targets, which are suitably used in the assay of cancer biomarkers determination. Various LDHs and their derived nanomaterials have been comprehensively employed in electrochemical sensing platform for *in-vivo* determination of cancer biomarkers.

4.1. Hydrogen peroxide determination

In biology and physiology, hydrogen peroxide (H_2O_2) has been considered as a gesturing molecule for accurate and prompt recognition of oxidative stress that can be associated with different kinds of fatal disorders. Its gigantic accumulation is dangerous to living organisms and it is triggered by mitochondrial functions, metabolic reaction involving enzymes as well as partially reduced oxygen in live cells [62]. It is worth mentioning that huge overproduction of reactive oxygen species can cause several enduring diseases which include aging, atherosclerosis, heart attack and cancer. However, H_2O_2 has key importance as industrial chemical that incredibly is used in various reactions, food and paper industries but its excessive production is tremendously detrimental to the living organisms. Consequently, it is crucially required to monitor exact levels of H_2O_2 associated with different living species to recognize its pathophysiological functions.

4.1.1. Enzymatic biosensor

The electrochemical determination of H_2O_2 is generally based on enzymatic sensors where the enzymatic electrode is fabricated by using different enzymes. In addition, two-dimensional layered inorganic solids, such as cationic clays and layered double hydroxides (LDHs) materials and their derivatives are the attractive sources for the development of sensor system since they have presented excellent biocompatibility, high chemical and physical stability and low production cost [63]. In this category of biosensor, LDH materials server as support to incorporate enzymes owing to superb intercalation features and exchangeability of interlayers anionic species. Bienzymatic configured material such as glucose oxidase (GOx) and horseradish

peroxidase intercalated together with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) in the interlayer region of ZnCr LDH has been used for H_2O_2 determination [64]. The superb electrical conductivity is presented between enzymatic redox centre of horseradish peroxidase and electrode surface by the redox-active ionic species intertwined in LDH layers. Ultimately, one would like to fabricate reagentless biosensor deprived of using any mediator with operating potential close to enzyme and this mediator free system is the featured improvement of this alleged third-generation of biosensors having high sensitivity. It is of great challenge to make feasible the direct-electron transfer between enzyme and electrode because the active sites of enzyme are completely buried by protein backbone and there is extended electron-tunneling between electrode and enzyme. Direct electron transporting in GOx intercalated MgAl LDH was reported first time by Zhang et al. [65] uncovering the new horizons for the development of so-called third-generation of biosensors. Wang et al. synthesized peroxidase-like MgAl-HCF LDH nanoflakes by facile hydrothermal approach and investigated its potential in electrochemical reduction of H_2O_2 with broad linear range. The electrocatalytic activity for H_2O_2 reduction was also evaluated by using the electrode modified by as-prepared MgAl LDH intercalated with hexacyanoferrate(III) (HCF) [66] and Prussian blue [67] which unveiled tremendous selectivity for H_2O_2 determination irrespective inhibition effect of dissolved oxygen. Compared with metal hexacyanoferrate materials, the as-prepared peroxidase-like LDH displayed enhanced stability in neutral as well as alkaline solutions and can be used as man-made mimics of enzyme in electrochemical biosensing systems. Unfortunately, these biosensors exhibit poor stability owing to the –OH ions released by the reduction of H_2O_2 which are capable to breakdown iron-cyanide bond. Some previous reports on enzymatic biosensors in which LDH layers intercalated with different bioconjugates such as myoglobin, [68] DNA, [69] hemin, [70] and horseradish peroxidase [71] have demonstrated high performances for selective and sensitive electrochemical determination of H_2O_2 molecule. Fig. 6. (a-f) has uncovered the process of the direct electrochemistry of HRP on HRP/C-Dots/LDHs/GCE. These enzymatic biosensors are benefitted with direct electron transferring, admirable sensitivity and selectivity originated from high catalytic activities of enzyme. However, the development of enzymatic biosensor faces the major challenges like inadequate loading of immobilized enzyme and feeble bio-stability due to nature of enzyme and consequently loses their bioactivity [72]. To overcome these problems, nanomaterials have been explored as mimic of enzymes which retain their bioactivity owing to

physicochemical/structural properties. Furthermore, simple LDHs and LDHs supported nanomaterials have demonstrated excellent affinity as well as sensitivity in H_2O_2 sensing platform compared with various heme protein immobilized LDH materials [14, 73].

Fig. 6. (a) SEM image and (b) XRD pattern of CoFe-LDHs, (c) cyclic voltammograms of GC, LDHs/GC, HRP/LDHs/GC, C Dots/LDHs/GC, and (e) HRP/C-Dots/LDHs/GC electrodes in 0.1 M PBS (pH¼7.0) at a scan rate of 50 mVs^{-1}, (d) CVs of the HRP/C-Dots/LDHs/GCE in 0.1 M pH 7.0 PBS solution in (a) 0 nM, (b) 2.0 mM, (c) 4.0 mM, and (d) 8.0 mM H_2O_2 at a scan rate of 50 mVs^{-1}, (e) The amperometric response of the HRP/C-Dots/LDHs/GCE to different concentrations of H_2O_2 from 0.1 μM to 23.1 μM in 0.1 M pH 7.0 PBS, the applied potential was-0.35 V, (f) the corresponding calibration curve of *I-C* obtained by chronoamperometry [71].

4.1.2. Nonenzymatic biosensor

The aforementioned enzymatic biosensors offered many advantages such as enhanced sensitivity, selectivity and low operating potential but still suffer the shortcomings of instability, complex immobilization and poor reproducibility. Nonenzymatic sensors have sparked increasing interests because of simplicity and promising capabilities towards *in vitro* tests. In nonenzymatic biosensors, LDH nanostructures act as electrocatalysts during redox processes. Moreover, noble metal inserted LDH nanomaterials with extended surface area, outstanding thermal and chemical stability, superb catalytic activity, and adequate biocompatibility are most preferably used in biosensing podium. Benefiting from the current advancement in nano-biotechnology, metal oxides entrapped LDHs have been the focus of rigorous research and can mimic the functions of natural enzyme to circumvent the common issues of enzymatic biosensors similar to easy denaturation, costly and prolonged preparation, and long-term instability. Several LDH materials providing stable matrix to incorporate noble metals within the layers such as Ag nanodentrites (NDs) on MgAl LDH [74] and Ag nanoparticles (NPs) inserted NiAl LDH [73] have been found to hold inherent peroxidase mimetic activity and have been used in H_2O_2 biosensing systems. The hierarchical architectures prepared by Ag NDs deposited on LDH substrate ensure open porous structure and high surface area that are capable to display enhanced electrocatalytic performance for H_2O_2 determination. Beside this, noble metal based nanomaterials possess some disadvantages of being rare, costly, easy to be poisoned and hazardous to atmosphere that limit their application as catalyst. Hence, there is substantial

interest to replace noble metals by some non-noble metals that are of economical, eco-friendly and good catalytic efficiency.

Recently, Ai's group modified GC electrode by Co intercalated LDH material [75] and Cu-MgAl LDH composites [76] calcined at 500℃ for 4 h, and the electrode showed striking performance when used as amperometric sensor. The ultrathin NiFe layered double hydroxide nanosheets (NiFe-LDHNS) were used as superior enzyme mimic peroxidase like activity towards colorimetric detection of H_2O_2 and glucose [77]. The proposed sensors exhibited intrinsic peroxidase-like electrocatalytic activity towards H_2O_2 reduction and its determination from flavored beverages. Porous material of metal-metal oxide frame-work mimics of peroxidase which has large surface area, good stability and less diffusion resistance than LDHs was obtained by thermal treatment. More recently, our group synthesized different morphologies of non-noble metals intercalated LDH nanostructures such as MnO_2-MgAl LDHs [8] and CuO-MnAl LDHs [25] by facile co-precipitation and hydrothermal methods and explored their practical applications as non-enzymatic H_2O_2 biosensing with detection limits of 5 µM and 0.126 µM as well as broad linear ranges of 0.05-78 mM and 6 µM-22 mM respectively. The monitoring H_2O_2 in biological systems was performed by electrodeposited gold nanoparticles (AuNPs) on an ITO electrode modified CoMn-LDHs with low detection limit. The results were also compared with CoAl-LDHs or MgMn-LDHs as support and it was concluded that the presence of binary transition metals in LDH layers may cause manifold interactions and nanoscale interfacial collaborations between AuNPs and CoMn-LDHs support [78].

Fig. 7. (a) Schematic illustration of NiMn-LDH/GO hybrids material (b and c) SEM and TEM images of NiMn-LDH/GO hybrids, (d) typical steady-state amperometric response to the injection of H_2O_2 for NiMn-LDH/GO at -0.45 V vs. Ag/AgCl. (Inset plots are amperometric responses vs. H_2O_2 concentration and enlargement of the area denoted by the small box) [79].

The well-ordered ultrathin inorganic-organic films of iron(III) porphyrin and CoAl LDH nanosheets were prepared *via* LBL assembly by Duan's group and its electrocatalytic potential towards the reduction of H_2O_2 was thoroughly studied [80]. Here iron(III) porphyrin served as a proficient mediator to expedite fast electron transport. A follow-up work by the same group was published based on ultrathin films of naphthol green B and LDH nano-platelets deposited on the

polished surface of GC electrode through electrostatic LBL self-assembly and its electrocatalytic behavior towards the determination of H_2O_2 was comprehensively studied [81]. Considering the proposal of this strategy, enzyme-free H_2O_2 sensing platform and several nanoscale electronic devices can be assembled by LBL inorganic-organic self-assembly. A detailed comparison of two CoAl LDH and NiAL LDH architectures was published by Yin et al [82]. Where NiAl LDH modified electrode exhibited quick heterogeneous electron transfer rate constants, lowest detection limits of 50 nM and 9 nM and improved reproducibility with RSD values of 3.2% and 1.8% respectively, while the sensor fabricated by CoAl LDH possessed wide linear range along with better sensitivity for H_2O_2 determination. The reduced electrocatalytic performance of CoAl LDH is may be due to the involvement of H_2O_2 in redox reactions CoAl LDHs on the surface of GC electrode. Particularly, highly insulating nature of metal oxides and LDHs is a big obstacle in the development of LDH based catalysts. Thanks to extraordinary intercalation features of LDHs and interlayer tunability of anions due to which this conundrum can be resolved by integrating LDH layers with conductive carbon substrates like carbon nanotubes (CNTs), conductive polymers or graphene oxide (GO). Heli et al. [83] synthesized nanocomposites comprising of hexagonal CoAl LDH nanoshales together with multiwall carbon nanotubes (MWCNTs) by reflux heating approach and H_2O_2 was electrocatalytically oxidized on the modified surface of electrode with high sensitivity because of synergy effect of enhanced electrocatalytic ability of LDHs as well as marvelous conductivity of MWCNTs. The layered assembly of NiMn-LDH/GO hybrids was prepared and employed as nonenzymatic sensor for sensitive determination of H_2O_2 as presented by Fig. 7. (a-d). The insertion of GO nanosheets in the interlayer galleries of LDHs greatly enhanced the electron transferring ability and promoted the distribution of active sites in hybrid material, which in turn increased the electrochemical performance of NiMn-LDH/GO hybrids modified electrode [79].

4.2. Hydrogen peroxide as cancer biomarker

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide, that are generated by univalent reduction of oxygen, mitochondrial functions, xanthine oxidase, and their concentration levels are associated with metabolism are considered as imported messenger for cellular signaling pathways [84]. ROS are detoxified by existing cellular antioxidants but imbalanced condition is discussed as oxidative stress exists [85, 86]. The maladjusted production of ROS causes oxidative stress which in turn acts as an integrator for

multiple processes triggering cardiovascular diseases, tumor growth and some other human mortalities because of free radical damaging of protein, lipids and nucleic acid in living systems [87]. Although, ROS contribute in intracellular transduction, abiotic anxiety influence, proliferation and development of cells but it is noteworthy that its massive production is exceptionally detrimental to living creatures. Particularly, plethora of cancer threats and cardiovascular risks are correlated with cell signaling through ROS production in living organisms [88, 89]. Previous reports have elucidated that living cancer cells can release significant amount of H_2O_2 after being stimulated by phobol 12-myristate-13-acetate (PMA) or N-formyl-methionylleucyl-phenylalanine (fMLP) [90].

Fig. 8. (a) and (b) SEM images of synthesized Mn5-MgAl with different magnification. (c) HRTEM of Mn5-MgAl with lattice fringes of 0.24 nm, 0.33 nm and 0.21 nm. (d) Amerometric i-t response of Mn5-MgAl with successive additions of different H_2O_2 concentrations in N_2 saturated stirring 0.1 M PBS (pH 7.4) at -0.8 V. Inset is the corresponding calibration curve of current vs. H_2O_2 concentration. (e) Bright field microscopy image of live HeLa cells. (f) Amperometric response of Mn5-MgAl GCE in 0.1 M PBS (pH 7.4) with the addition of 10 mM fMLP in the absence (upper) and presence (lower) of HeLa cells [8].

Consequently, being a promising biomarker, it is quite necessary to monitor the exact level of ROS which is conspicuous candidate for diagnosis of cancer and other diseases as well as an important pathway for reliable understanding of pathological, physiological and biomedical role of multi-functioning ROS [91, 92]. Therefore, perseverance struggles are being made to develop new analytical approaches and superb performing instruments to secure humankind from chronic diseases. The non-precious metal decorated LDHs nano-architectures (MnO_2-MgAl) was employed to construct highly sensitive nonenzymatic electrochemical biosensor to detect H_2O_2 triggered by live human cervical cancer cell lines (HeLa) upon being stimulated by fMLP as shown in Fig. 8 [8] Because of excellent intercalation features of LDHs, MgAl LDH's host layers entrapped considerably enhanced mass percent of MnO_2 NPs that endow abundant electroactive sites for H_2O_2 redox. The relevant low detection limit encouraged its reliable usage in real-time monitoring of H_2O_2 secreted by live HeLa cells. Additionally, metal oxides such as CuO intercalated with MnAl LDHs nanospheres hybrid material with their unique structural as well as synergistically combined multifunctional properties are expected for various important electrochemical applications due to their inherent p-n junction characteristics. Due to enhanced

electrocatalytic activity towards H_2O_2 reduction in terms of low real detection limit and wide linear range, the practical application as-prepared CuO@MnAl NSs based biosensor has been explored in real-time monitoring of H_2O_2 released by live tumor cells and normal cells as well (Fig. 9) [25].

Further depending upon the amount of H_2O_2 secreted by each of cell lines, it is concluded that the flux of H_2O_2 depend upon different types of cell lines. It is well documented that cancerous cells proliferate quickly because of rapid oxidative metabolism to engender more H_2O_2 efflux. Therefore, the proposed biosensor has enough sensitivity to make solid differentiation between tumorigenic and non-tumorigenic cells. Moreover, Hammami et al. [93] explored NiAl LDH material based screen printed electrode (SPE) immunosensor to investigate macrophage mannose receptor (MMR, CD206) as cancer inflammatory profiles. The particular association between antibody (anti-MMR) modified economical electrodes and the rHu-MMR so called CD206 or MRC1 have been exhibited for the concentrations less than 10.0 ng/mL with a detection limit recorded to be lower than 15 pg/mL. Table 1 lists a series of LDHs based electrochemical biosensors developed in the last three years for H_2O_2 determination.

Fig. 9. Electrochemical sensing mechanism of LDHs hybrid nanosphere, (a) SEM-EDX mapping of CuO@MnAl NSs, (b) Bright-field images of MCF7 cells used in real-time tracking of H_2O_2, Amperometric responses of CuO@MnAl NSs/GCE in 0.1 M PBS (pH 7.4) upon (c) the inoculation of 10 µL fMLP (0.1 mM) in the presence of MCF7 cells and (d) an aliquot addition of serum followed by successive addition of 20 µM H_2O_2 in stirring PBS [25].

Table 1 Analytical performance parameters of several current LDH nanostructures based electrochemical biosensors for H_2O_2 determination.

Electrode materials	Linear range (mM)	Detection limit (nM)	Sensitivity (µA mM^{-1} cm^{-2})	Real sample	Ref.
Hemoglobin immobilized ZnAl LDH	0.99×10^{-5}~ 15.8×10^{-5}	5.4	40.63	-	[94]
HRP immobilized on carbon nanodots/CoFe LDH	0.0001~0.023	40	470	-	[71]
Hexagonal CoAl LDH nanoshales/CNTs composite	0.1~4	10000	118	Different water	[83]

CoFe LDH nanoplates	1×10^{-5}~ 3×0^{-3}	5	-	Water and milk	[95]
Au NPs electrodeposited on CoMn LDH	0.0001~ 1.27	60	125.0	Human serum	[78]
NiAl/LDH/Ag NPs composite	0.01~10	600	1.863	Oxidizing agent of hair dye	[73]
MnO_2-MgAl LDHs nanohybrids	0.05~78	500	0.9352	HeLa cells	[8]
CuO@MnAl LDHs nanospheres	0.006~22	120	-	MCF-7 and U87 cells	[25]
Core-shell Fe_3O_4@CuAl LDH nanospheres	0.0001~10	1	-	H-22 and BEL-7402 cells	[14]
N-doped Graphene/CoFe NPs	0.00~8.6	28	435.7	Serum and HeLa cells	[96]
NiFe LDHs nanosheets on Ni foam	0.0005~0.84	500	1704	-	[97]
NiMn LDHs on graphene oxide	0.02~5.8	4400	96.82	Bovine serum	[79]
Chitosan/2D ZnAl LDH composite	0.02~6	12400	220.4	-	[98]

4.3. Glucose determination

In living systems, small biological molecules consisting of hydrogen peroxide and glucose with their definite diagnostic concentrations act as biomarkers for pointing out numerous diseases. The electrochemical enzymatic glucose biosensors fabricated by various nanohybrid materials generally demonstrate magnificent performance in terms of low detection limit, wide range, good stability resulting from synergistic combination of different components and hybrid materials. These biosensors are extremely necessary for *in vitro* determination of glucose in human biological fluids. Owing to global ever growing population with clinical chronic conditions of diabetes mellitus which is more commonly known as diabetes, glucose biosensors are of crucially required now-a-days. Clinical diagnosis of diabetes has revealed irregularity in blood glucose levels, occurred by malfunctioning of pancreatic β cells, liable of insulin

generation that regulate blood glucose in body and metabolic disorderness connected with several macro and micro vascular sequelae [99]. Some lethal diseases such as nervous, cardiac, renal, ocular diseases are closely associated with elevated levels of blood glucose threatening the life completely [100]. There should be intensive care about monitoring of blood glucose level in patients because millions of individuals are suffering diabetes and it has been predicted that the figure for diabetes patients would be more than twofold of the recent value globally [101]. According to the current projections, diabetes will be the 7th major source of death as of 2030 [102] and worldwide diabetes occupants increasing to 590 million by 2035 [103]. Therefore, diabetes being positioned at top five among chronic diseases, everybody in the world requires persistent monitoring of blood glucose.

4.3.1. Enzymatic biosensor

The enzymatic glucose biosensors based various nanomaterials probably demonstrate good performance like low detection limits, high sensitivity, selectivity, stability and reproducibility. These are of substantial importance for *in vitro* glucose determination from biotic fluids. The electrochemical biosensing platform based on GC electrode modified by inorganic-organic hybrid LDHs materials such as glucose oxidase (GOD) immobilized on Zn_3Al-Cl LDH, [104] ZnCr-FcPSO_3, [105] Ni^{2+}/MgFe LDH, [106] and NiAl-NO_3 [107] *via* coprecipitation and electrodeposition approaches respectively has been investigated for glucose determination. GOD is entangled in between the host layers of LDHs by electrostatic interactions as well as cross-linking with glutaraldehyde vapors to control enzyme release. The inserted anions played the role of mediators in shuttling electrons between enzyme and electrode surface. The synergy of marvelous biocompatibility of GOD and good properties as well as charge transport ability of LDHs gave rise to a biocomposite film modified electrode presenting superior performance for glucose determination. The majority of the well-known amperometric glucose biosensing systems are based on immobilization of definite oxidase (i.e., GOD) and electrochemical measuring of enzymatically released H_2O_2 efflux. The oxidase-based biosensors need comparatively high applied potential for effective oxidation of H_2O_2 opening up the opportunities for biological co-existive model molecules like uric acid (UA), ascorbic acid (AA) and 4-acetaminophen (AP) to be electrochemically oxidized triggering interference in the sensing of glucose. Benefitting from the recent advancement in nanotechnology and biotechnology, nanomaterial based artificial enzymes -"nanozymes"- such as cyclodextrins,

metal complexes, porphyrins and nonenzymatic electrochemical biosensors have captivated immense current research enthusiasm [108].

Fig. 10. (a) Schematic illustration of the electrocatalytic oxidation mechanism of glucose. (b) SEM images of the as-prepared NiAl LDH product. (c) CV curves of the NiAl LDH/Nf/GCE in 0.1 M NaOH solution with increasing glucose concentration from 1 to 100 μM [109]. (d and e) SEM images of NiCo LDH/carbon cloth composites under different magnification. (f) Amperometric sensing of glucose by the successive addition of glucose for the as-prepared LDH and its composites at 0.5 V in 0.1 M KOH. Inset is the current response before and after addition of glucose [110], (g) Schematic illustration of glucose electrooxidation on NiFe-LDH/NF electrode surface in alkali solution, (h) high-magnification SEM images of NiFe-LDH supported on Ni foam, (i) NiFe-LDH electrode and $Ni(OH)_2$ electrode with the successive addition of glucose to NaOH solution per 25 s at 0.50 V [111].

Nanozymes can mimic the morphologies and functionalities of naturally occurring enzymes. This provides us impetus to take the challenge of constructing electrochemical sensors that can avoid the usually encountered riddles of enzymatic sensors such as easy to be denatured by environmental changes, time-consuming and expensive fabrication procedure, and inadequate long-term stability. Shao et al. [112] constructed ultrathin films based on CoFe LDH nanoplatelets and manganese porphyrin anion as building blocks on ITO substrate by magnetic field assisted (MFA) layer by layer assembly. The electrode modified by alternatively heteroassembled superlattice type material demonstrated surged electron transport process and incredible electrocatalytic ability towards glucose sensing with low detection limit and good resistance again poisoning in chloride ion solution.

4.3.2. Nonenzymatic biosensor

There is an immense growing interest in nonenzymatic sensors based the direct electrocatalytic oxidation or reduction of small molecules due to their improved stability, simple synthesis approach, and demonstrating a promising strategy towards sensing platform for *in vitro* experimental assays [113]. Tow principle parameters are shadowed during nanomaterial based nonenzymatic detecting of glucose: firstly, the electro-oxidation process of glucose is a kinetically controlled sluggish reaction and secondly, rather than geometric area, the nanoscopic surface area of electrode which is also called real surface area, control the accompanying faradaic currents. In contrast, the electro-oxidation of interferences is diffusion controlled route as well as their faradaic currents are dependent on actual geometric area of electrode and

independent to the roughness feature. Consequently, the nanomaterials holding huge surface area and enhanced roughness factors can be accountable of increasing faradaic currents for glucose oxidation and reduce the effect of interfering electroactive species such as AA, UA and AP at similar time [114]. In this regards, LDHs are promising class of nanomaterials with enlarged surface area and roughness factor. [115] Particularly, nickel containing LDHs such as NiAl LDHs with chitosan composites as depicted by Fig. 10 (a-c) [116], NiAl LDHs nanosheets grown on Ti substrate, [117] NiAl LDH/nafion, [109] and CoAl LDHs [118] have been synthesized by facile coprecipitation and electrodeposition methods as well as applied for nonenzymatic glucose sensor. Apart from most of GOD based glucose biosensors, the aforementioned biosensors detect glucose in the absence of GOD catalase because one most important phenomenon of electro-oxidation mechanism of Ni ions occurring in NiAl LDHs where Ni(III) centers react as oxidant. Furthermore, some simple LDH materials have been employed in electrocatalytic oxidation of glucose in their own rights without coupling with conductive substrates. For instance, Nickel–Iron layered double hydroxide (NiFe-LDH) ultrathin nanosheets with porous structure on Ni foam [111] as well as NiFe LDHs for micro liquid-phase extraction to detect glucose in bananas [119] were synthesized that performed as peroxidase mimics. These binder-free LDH materials presented good sensing performances due to the synergistic effect of abundant active sites, large specific surface area and quick diffusion of the electrolyte.

The composition of nanomaterials is another critical parameter that affects performance of nonenzymatic sensors. Electrochemical determination of glucose was carried out using NiAl-layered double hydroxide (NiAl-LDH) incorporated carbon microcylinder (CMC) hybrid employing typical carbon based microelectromechanical systems (C-MEMS) techniques together with in situ growth progress and showed satisfying results due to good conductivity of carbon material coupled with Ni centers [120]. The intertwining of nickel hydroxide nanosheets with highly-aligned carbon nanotube scaffold was completed and fabricated material exhibited reasonable performance in non-enzymatic glucose sensing [121]. Noble metal incorporated LDHs self-assembled nanostructures such as ZnAl-NO_3 LDH/Au NPs LBL assembly [122] and Au NPs decorated NiAl LDH/SWCNTs/graphene composites [123] with improved electron transfer kinetics are extensively used catalysts owing to their enhanced electrocatalytic ability towards glucose oxidation, inherent conductivity due to electron tunneling junctions resulting

from the interlayer plasmon coupling as well as 3D intertwined CNTs-graphene network and chemical inertness. The activity and stability of nanocatalysts can also be enriched by immobilizing them onto the active support of carbon materials such as CNTs, conductive polymers, graphene and their composites because of intriguing properties of these carbon based materials such as enlarged surface area, abundant surface active sites and high stability. Table 2 lists a series of LDHs based electrochemical biosensors developed in the last three years for glucose determination.

Graphene, as π electron rich carbon support has been inserted into LDHs structures in order to overcome the semiconductive nature of LDHs materials which in turn boosts up their conductivity. As a proof of concept, nickel iron layered double hydroxide/graphene nano composites [124] and NiCo layered double hydroxide (LDH) nanosheets/graphene nanoribbons [125] were synthesized by electrochemical deposition and coprecipitation respectively to study their electro-oxidation capability. These modified electrodes demonstrated high diffusion coefficient as an electro-catalysts as well as superb performance in electrochemical sensing of glucose. The nanocatalysts based on the graphene quantum dots and CoNiAl LDH [126] and NiCo LDH nanosheet array constructed on carbon cloth [110] have been fabricated by one-pot coprecipitation approach and applied for the direct electro-oxidation of glucose as shown in Fig. 10 (d-i). The resultant enhanced electrocatalytic activity of nonenzymatic glucose sensors is due to synergistic effect of interconnected 3D structure of carbon support with abundant electrochemical active surface areas, accelerated charge transfer rate and nanoscale interface.

Table 2 Analytical performance parameters of several current LDH nanostructures based electrochemical biosensors for glucose determination.

Electrode materials	Linear range (mM)	Detection limit (μM)	Sensitivity (μA mM^{-1} cm^{-2})	Real sample	Ref.
Co/Al layered double hydroxide coated electrode	1.0×0^{-2}~ 0.0376	9	-	Cereals	[118]
Au NPs-NiAl LDH-CNTs/graphene composites	0.010~6.1	1	1989	Human serum	[123]
Graphene QDs/CoNiAl-LDH nanocomposites	0.01-14.0	6	48.717	Fruit juices	[126]

NiAl LDH networks by C-MEMS techniques	0.2~ 18.6	120	125	Human serum	[120]
3D NiCo nanosheets array/carbon cloth	0.001~ 1.5	0.12	5120	Human serum	[110]
3D ultrathin NiFe LDH nanosheets	Upto 0.8	0.59	3680.2	Human blood	[111]
Graphene/NiFe LDH nanocomposites	-	240	389.7	-	[124]
$Ni(OH)_2$/CNT fiber microelectrode	0.02~ 10.5	0.645	1220	Human serum	[121]
NiCo LDH nanosheets/graphene nanoribbons	0.005~ 10	0.6	344	-	[125]
NiFe LDH nanocomposites	0.001~ 5.7	0.25	-	Bananas	[119]
CuAl layered double hydroxides	0.0001~ 0.24	0.02	-	Human blood	[127]
1D Ag nanowire/NiCo LDH	0.002~6	0.66	71.42	Human serum	[128]
NiMn LDH on graphene oxide	0.002~3.386	1.2	839.2	Bovine serum	[79]

4.4. Dopamine determination

Dopamine (DA), a monoaminergic molecule, is one of the important family members of catecholamines which are associated with family of excitatory chemical neurotransmitters. It plays crucial roles in the mammalian central nervous system, cardiovascular, hormonal systems and certain intractable psychiatric diseases, such as schizophrenia and Parkinsonism. As one of the extensively studied monoaminergic neuroreceptors, DA holds immense significance in modulating various features of the brain circuitry of mammals [129]. Thus there is an emerging requirement to establish highly sensitive and selective approaches for direct measurement of DA, since it has been acknowledged as chemical messenger molecule in central nervous system. The electrochemical biosensors based on nanoscale materials such as LDHs and their derivatives are

attracting considerable attention of researchers for biological applications [130]. Carbon ionic liquid electrode coated by NiAl LDH [131] and glassy carbon electrode modified with gold NPs and organophillic LDH [132] have been fabricated and exhibited improved electrochemical response towards DA determinations. The improved electrochemical performance of modified electrode is owing to excellent ionic conduction of binder, reduced resistance of coated electrode, enhanced ion exchange properties and intrinsic catalytic ability of ionic liquids. To enhance the electrocatalytic activity of LHDs, Poly-o-phenylenediamine intercalated MgAl LDHs [133] was fabricated via simple one step hydrothermal approach and used in sensitive determination of DA. Fig. 11. demonstrates that hydrothermal method was employed to prepare flower like morphology of amorphous $Ni(OH)_2$ nanoboxes/nickel–cobalt layered double hydroxides (NiCo-LDHs) [134] and the electrode modified by as-synthesized nanocomposites presented superior electrocatalytic efficiency towards DA oxidation with low detection limit of 17 nM. The intriguing sensing ability may possibly be due to larger surface-to-volume ratio which endowed more active sites.

Fig. 11. (a) Schematic illustration for the preparation of $Ni(OH)_2$ nanoboxes, (b and c) SEM and TEM images of $Ni(OH)_2$/NiCo-LDHs nanocomposites, (d) Typical amperometric response of the $Ni(OH)_2$/NiCo-LDHs/GCE on successive addition of DA into the stirring N_2-saturated 0.1 M PBS (pH7.5) at an applied potential of 0.22 V [134].

Moreover, inspired by inorganic/organic nanohybrids of LDHs through the process of its exfoliation into single nanosheet building blocks, LBL alternate assembly of positively charged MgAl LDHs and negatively charged cobalt phthalocyanine grown on ITO substrates [135] and inorganic-chromophore host-guest nanocomposites such as Zn_2Al LDH/FLU-HES *via* coprecipitation have been synthesized. The electrode modified by MgAl LDH/CoPcTs exhibited superb electrocatalytic performance for DA oxidation which is related to the Co(II)/Co(III) couple in the MgAl LDH/CoPcTs as well as immobilization of electroactive CoPcTs molecules. Because of inherently feeble conductivity and to further increase the sensing performances of LDH based materials; intrinsically conductive graphene has been intercalated between interlayer regions of LDHs. The fabrication of superlattice type nanocomposite of NiAl LDH/graphene *via* conventional coprecipitation method and subsequent reduction of graphene oxide is reported Evans's group [136] and its electrochemical properties for electro-oxidation of DA molecule

have been investigated. The electrode modified by NiAl LHD/G demonstrated extremely higher redox capability than those of pristine NiAl LDH. It can be concluded that Ni centers in NiAl LDH/G nanohybrids hold enlarged electro-active surface area as compared with pristine NiAl LDH.

4.5. Other biomolecules determination

Several other biomolecules for instance ascorbic acid (AA) and uric acid (UA) have also been detected by developed biosensors, in particular using LDHs as electrode material. AA with antioxidant property holds a key role in contributing various biological functions and obstructing several chronic diseases such as mental illness, infertility, AIDS and cancer. Moreover, AA is also used as an antioxidant in all food stuffs, animal feed as well as cosmetic industries. Because of AA concentration being at milli molar level in animal central nervous system, it is necessary to develop reliable, simple and sensitive assays for the measurement of AA with high accuracy for diseases diagnostic purposes and food safety applications [137]. An inorganic-organic composite ultrathin films of naphthol green B (NGB) with NiAl LDHs nanoplatelets [138] and nickel hexacyanoferrate (NiHCF) with NiAl LDHs [139] were fabricated by layer by layer assembly and electrodeposition method respectively. The direct electrochemistry behavior of both modified electrodes exhibited striking electrocatalytic ability for the oxidation of AA in PBS solution. Compared with NiHCF-NiAl LDHs, ITO electrode modified with NGB-NiAl LDHs presented ultrasensitivity with low detection limit owing to effectively immobilized NGB molecules in LDH layers as well as reversible one-electron redox process of the Fe^{III}/Fe^{II} couple in NGB which in turn accelerated the electron transport between NGB and electrode surface. UA, an important biomolecule which exist in human urine and blood is the key product of purine metabolism. The abnormalities in UA level can trigger certain lethal diseases like gout and hyperuricaemia. Additionally, it is well-documented that UA is promising and authenticated premotor of Parkinson's disease (PD) biomarker which may assist in prognosis and progression of PD. Consequently, it is necessary to measure exact levels of UA in human blood serum. The surface of glassy carbon electrode was modified by thin film of ZnAl LDHs [140] by electrodeposition method and demonstrated improved electrocatalytic currents and well-separated oxidation potentials for simultaneous determination of epinephrine (EP) and uric acid (UA), when implemented as electrochemical sensor. Simultaneous determination of nanomolar level epinephrine and acetaminophen on a glassy carbon electrode modified with MgAl LDHs-

nickel hydroxide nanoparticles-multiwalled carbon nanotubes composite material has been performed by Ali Babaei [141]. The proposed biosensor was practically employed in the determination of these biomolecules from human urine and blood serum samples. The results validated that the modified electrode holds intriguing properties such as low cost, high sensitivity, selectivity, and reproducibility which show its usability both in research as well as clinical laboratories.

Table 3 Comparison between LDH nanostructures and other materials based electrochemical biosensors for biomolecule detection

Electrode materials	Linear range (mM)	Detection limit (nM)	Electrode materials	Linear range (mM)	Detection limit (nM)	Analyte	Ref.
ZnAl LDH	0.99×10^{-5}~ 15.8×10^{-5}	5.4	MoS_2-PtNP	0.02~4.72	345	H_2O_2	[94, 142]
CoFe LDH	1×10^{-5}~ 3×0^{-3}	5	Cu_2O-GNP	-	34	H_2O_2	[95, 143]
Fe_3O_4@CuAl LDH	0.0001~10	1	PDDA-RGO/MnO_2/Au	0.1~1.3	600	H_2O_2	[14, 144]
CoFe LDH	0.00~8.6	28	MoS_2/ GR composites	0.0062~0.225	1250	H_2O_2	[96, 145]
3D NiCo LDH	0.001~1.5	120	Au-Pd/MoS_2	0.0008~10	4×10^5	Glucose	[110, 146]
NiCo LDH	0.005~10	600	WSe_2/GOx/GTA	0.077~0.274	52000	Glucose	[125, 147]
NiFe LDH	0.001~5.7	250	PDDA-RGO/MnO_2/Au	-	1800	Glucose	[119, 148]
CuAl LDH	0.0001~0.24	20	GOx-GO-MoS_2/GCE	2.0~20	29×10^5	Glucose	[127, 149]
NiCo LDH	up to 1.08	17	3D rGO-Co_3O_4 nanograins	0.001~0.03	277	DA	[134, 150]
NiAl LDH	0.0001~0.097	2	PABSA-rMoS_2	0.001~0.05	220	DA	[151, 152]

4.6. Improvements over conventional techniques

Up till now, multiple methodologies have been employed for quantitative detection of different analytes together with fluorescence, chemiluminescence, electrochemiluminescence and spectrophotometry. Fluorescence signals can be produced by assessing either their intensity or lifetime (normally $\leq 10^{-5}$ s). Thus, it could provide evidence of biomolecules structure and response mechanism of analytes in diseased versus healthy conditions. High-performance liquid chromatography (HPLC) coupled with mass spectrometer [153], atomic emission or absorption spectroscopy, surface-enhanced raman scattering (SERS), surface plasmon resonance (SPR) and colorimetric approaches have also been established [154]. Numerous colorimetric techniques have been engaged for *in vitro* detection of biomarkers from human serum matrices. SERS has been well-acknowledged as an imperative analytical method for non-invasive, non-limited and sophisticated sensing of analytes at low concentrations. For example, field-effect transistor (FET) biosensors possess high performance which can deliver real-time detection of metabolite molecules at trace levels [155]. Majority of these approaches are very advanced and highly dedicated but still hold few shortcomings such as costly inputs, complex responsive edifices and also demand for expert technicians. Electrochemical biosensors have been well-renowned in clinical diagnosis owing to their beneficial features over traditional analytical approaches.

The *in vitro* detection of small metabolic molecule and reactive oxygen species is of great significant for screening, monitoring and diagnosis of cancer and other related diseases. So, any detection method should be unequivocally ASSURED (affordable, specific, sensitive, untroublesome, rapid and robust, equipment-free and deliverable) for *in vitro* quantification and detection of targets from the complex biological components to meet the requirements of early diagnosis of chronic diseases. Wide linear range, low detection limit, fast response time, high sensitivity and selectivity towards the target are the important aspects needed for *in vitro* quantification of small metabolites. Consequently, application of electrochemical biosensors in medical research can deliver reliable answer for rapid, accurate, economical, environmental friendly and programmed multianalyte analysis [156]. Unalike optical sensing systems, the compassion of electrochemical biosensors are not decreased by reducing their magnitudes, contributing exciting prospects for emerging transferrable devices.

For electrochemical determination of biological molecules, enzymatic and non-enzymatic biosensors have been established. Nonetheless, the enzymatic biosensors suffer from some

intrinsic drawbacks including short life-time, complexity in immobilization process, instability, easy to be destroyed that obstruct their usability [157]. Consequently, electrochemical biosensors have always been an unambiguous investigation into nanomaterial based biosensors that clutch excellent electrocatalytic aptitude, high selectivity and anti-interference ability with low cost of health care services. The primary component of electrochemical sensor is the working electrode; therefore its modification with functional nanomaterials such as LDHs will significantly enhance the overall performance of electrochemical biosensors.

5. Conclusion and future perspective

The fascinating LDH nanoarchitectures represent one of the most technologically promising materials as a result of being cost effective, relatively easy preparation, and several composition/preparation variables that might be implemented. In this article, we have comprehensively summarized the recent advancement in LDH and LDH based nanomaterials as electrocatalysts and biosensors for determination of small metabolic molecules, in particular when they are implemented in the ultrasensitive *in vitro* determination of emerging biomarker, i.e., H_2O_2 concentrations in living cells, blood glucose level as well as dopamine efflux for the diagnostics and treatment of cancer, diabetes and other chronic diseases respectively. Consequently, there are six noteworthy goals of this review; (i) various effective strategies have been well-emphasized for the synthesis of LDHs nanomaterials and their derivatives with high crystallinity, controlled size, as well as interior tunability, which are highly required for practical applications; (ii) the potential usage of LDHs based materials in electrocatalysts; (iii) to differentiate electrochemical enzymatic and nonenzymatic biosensors; (iv) to identify the progress in hydrogen peroxide, glucose, dopamine and others small molecules biosensor performance as a result of employing layered double hydroxide based nanomaterials; (v) to evaluate useful concentration ranges of glucose and hydrogen peroxide detected by using these biosensors for real analytical circumstances; (vi) to distinguish tumorigenic and nontumorigenic cell lines *via* measuring the triggered H_2O_2 efflux after being stimulated from living cells by certain enzyme. It has been demonstrated that the attractive properties like physiochemical, mechanical, facile ion exchangeability and tunability of these nanomaterials have improved the sensitivity and selectivity of electrochemical biosensors that can be used towards *in vitro* determination of emerging biomarker.

With the innovation of nanotechnology, the sensing performances of electrochemical biosensors towards emerging biomarkers will further be improved by the development of nanomaterials, making them more reliable for *in vitro* diagnostics. The utilization of LDHs based nonenzymatic sensors can circumvent the most common issues of enzymatic sensors including instability and highly expensive, signifying a favorable strategy towards the development of practically accessible sensing platform for *in vitro* diagnosis of diseases. We further predict that with the advances as well as revolutions in LDH nanomaterial based biosensors, the performances of biosensing devices such as sensitivity, selectivity, good reproducibility and ease in operatability, will be more improved opening up new avenue for extremely effective and reliable detection of cancer as well as other biomarkers in biomedical applications.

NOTES

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Highlights

- Various synthesis approaches to fabricating different LDHs nanoarchitectures
- The electrochemical performances of LDHs have been evaluated
- Determination of H_2O_2, glucose, dopamine and other biomolecules has been studied
- Real-time monitoring of biomarkers from blood serum, urine and secreted by various live cancer cells

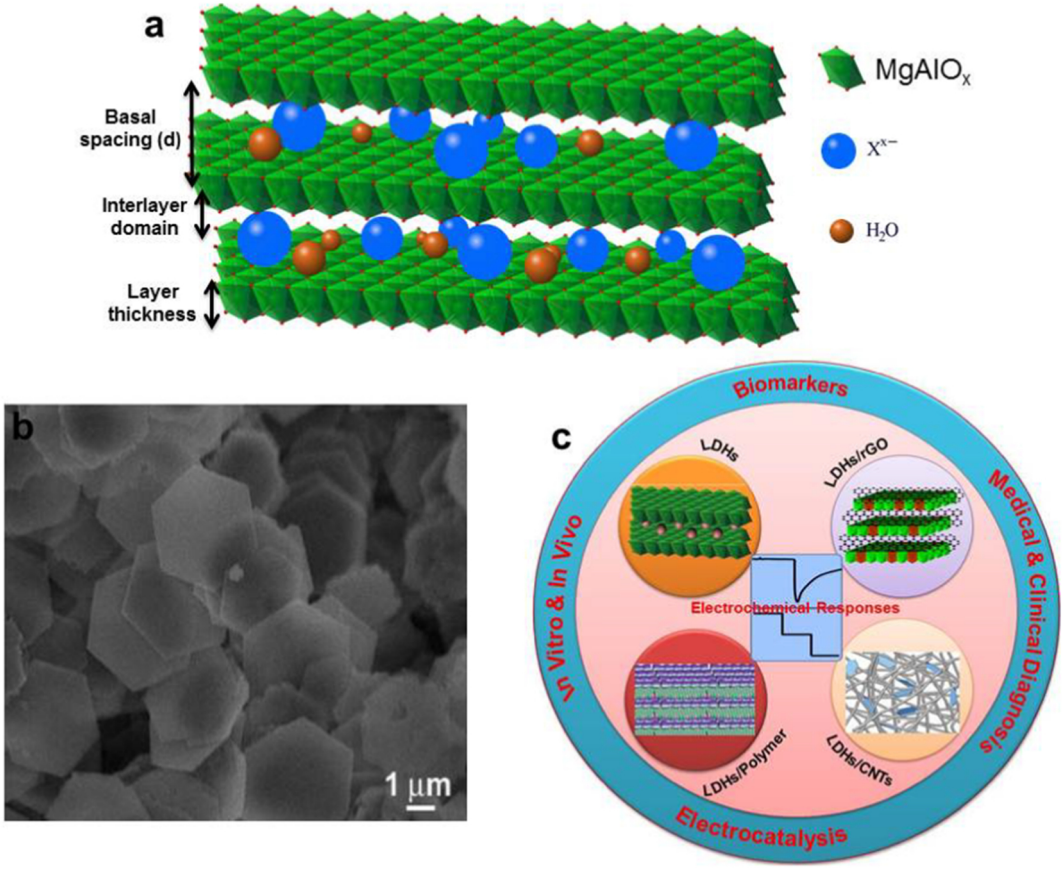
a
Basal spacing (d)
Interlayer domain
Layer thickness
MgAlO$_x$
X^{x-}
H_2O
b
1 μm
c
Biomarkers
Medical & Clinical Diagnosis
Electrocatalysis
In Vitro & In Vivo
LDHs
LDHs/rGO
Electrochemical Responses
LDHs/Polymer
LDHs/CNTs

Figure 1

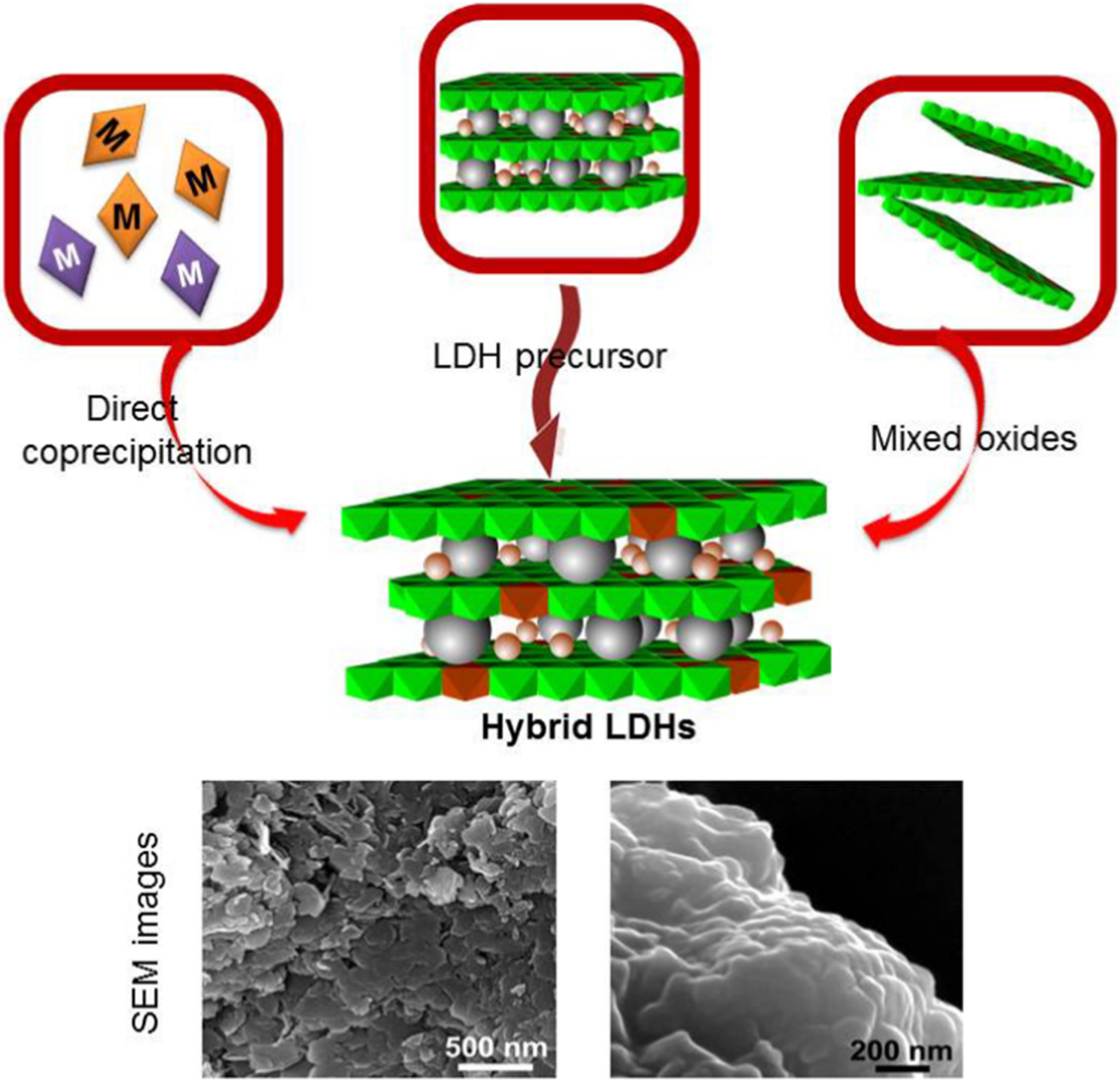
M
M
M
M
M
LDH precursor
Direct
coprecipitation
Mixed oxides
Hybrid LDHs
SEM images
500 nm
200 nm

Figure 2

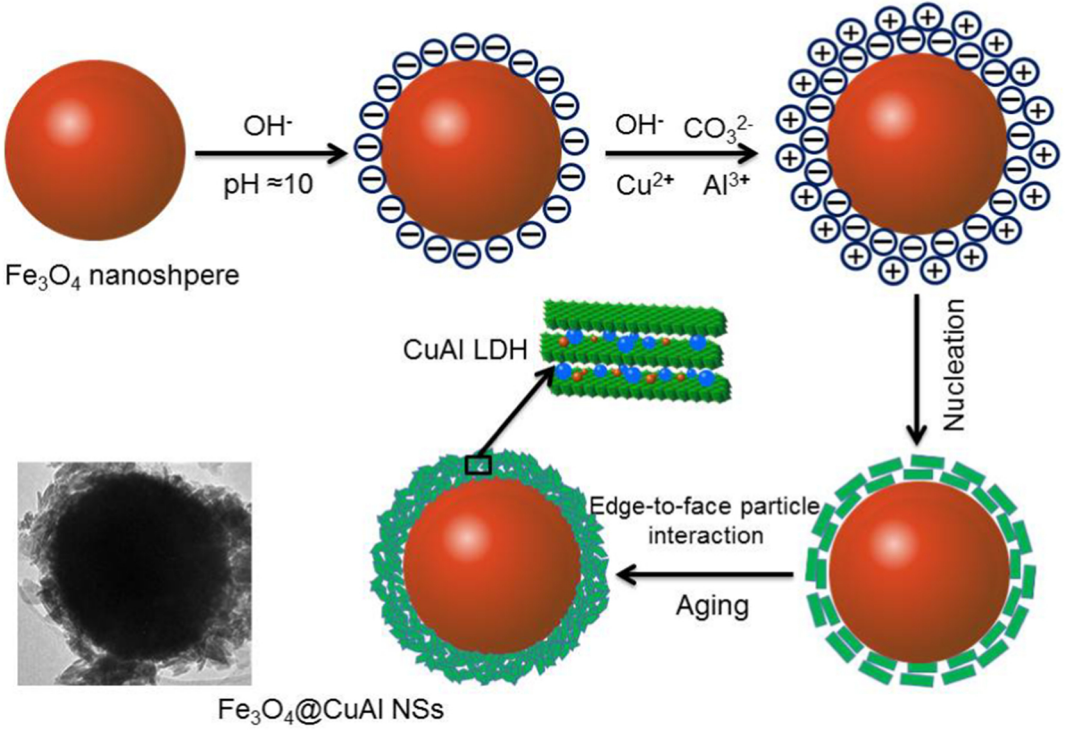
OH-
pH ≈10
OH- CO3 2-
Cu2+ Al3+
Fe3O4 nanoshpere
CuAl LDH
Nucleation
Edge-to-face particle interaction
Aging
Fe3O4@CuAl NSs

Figure 3

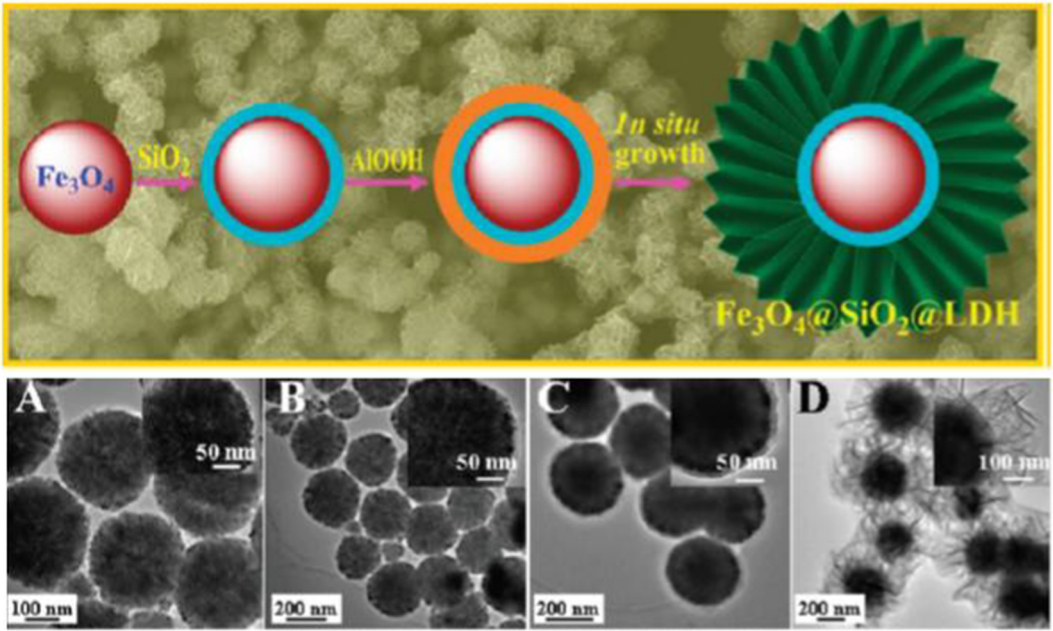
Fe3O4
SiO2
AlOOH
In situ
growth
Fe3O4@SiO2@LDH
A
50 nm
100 nm
B
50 nm
200 nm
C
50 nm
200 nm
D
100 nm
200 nm

Figure 4

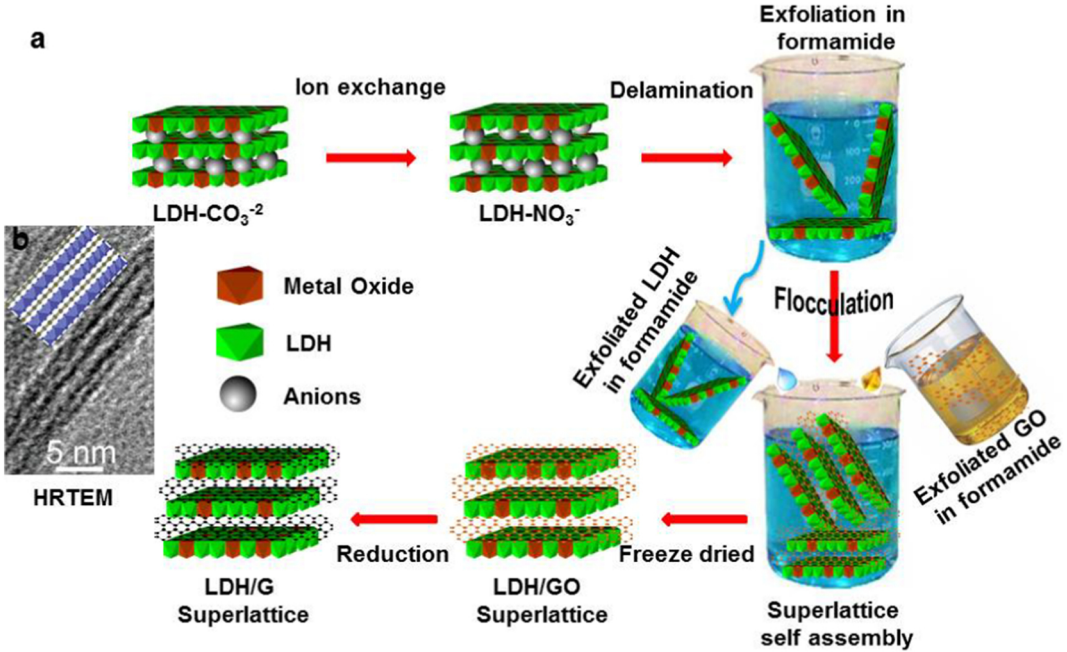
a
Ion exchange
Delamination
Exfoliation in formamide
LDH-CO_3^{-2}
LDH-NO_3^-
b
Metal Oxide
LDH
Anions
5 nm
HRTEM
Exfoliated LDH in formamide
Flocculation
Exfoliated GO in formamide
Reduction
Freeze dried
LDH/G Superlattice
LDH/GO Superlattice
Superlattice self assembly

Figure 5

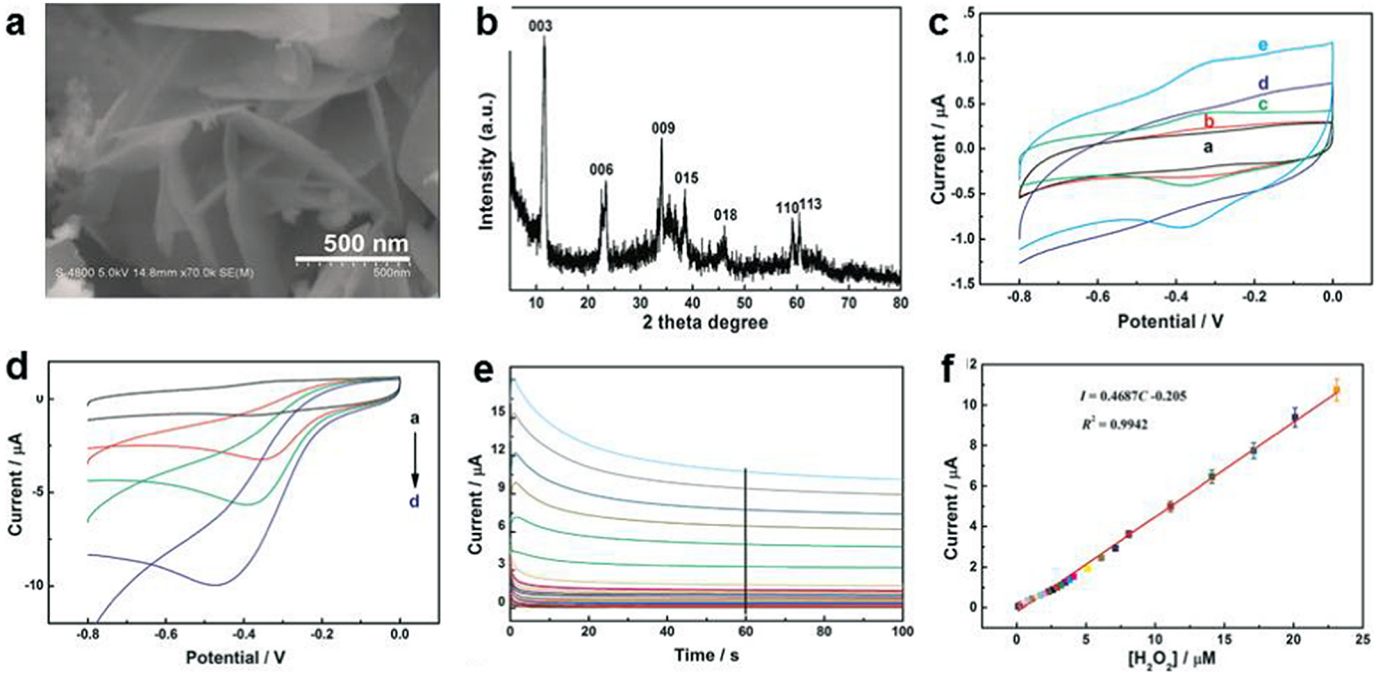
a
500 nm
S-4800 5.0kV 14.8mm x70.0k SE(M)
500nm
b
003
006
009
015
018
110
113
Intensity (a.u.)
2 theta degree
c
e
d
c
b
a
Current / μA
Potential / V
d
a
d
Current / μA
Potential / V
e
Current / μA
Time / s
f
I = 0.4687C -0.205
R² = 0.9942
Current / μA
[H2O2] / μM

Figure 6

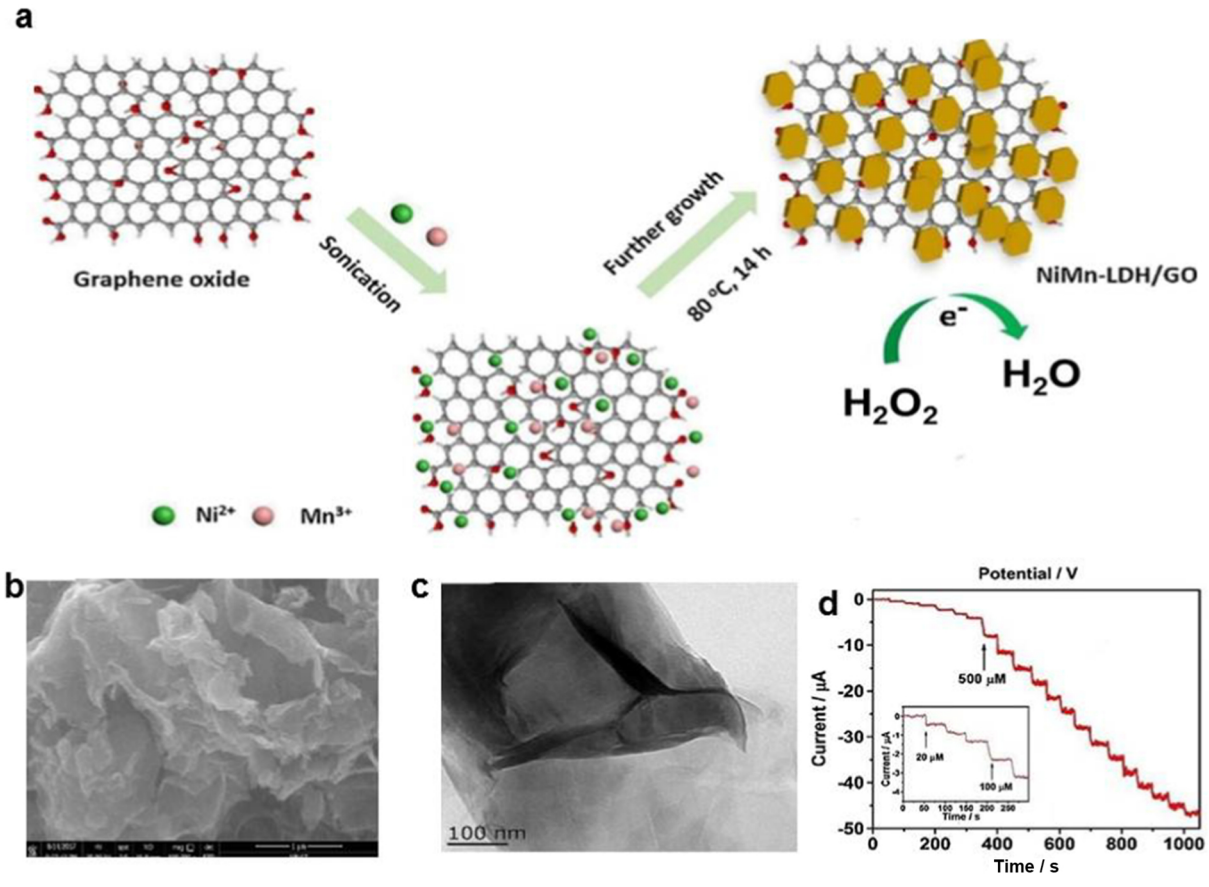
a
Graphene oxide
Sonication
Further growth
80 °C, 14 h
NiMn-LDH/GO
e-
H2O2
H2O
Ni2+
Mn3+
b
c
100 nm
d
Potential / V
Current / μA
500 μM
20 μM
100 μM
Time / s

Figure 7

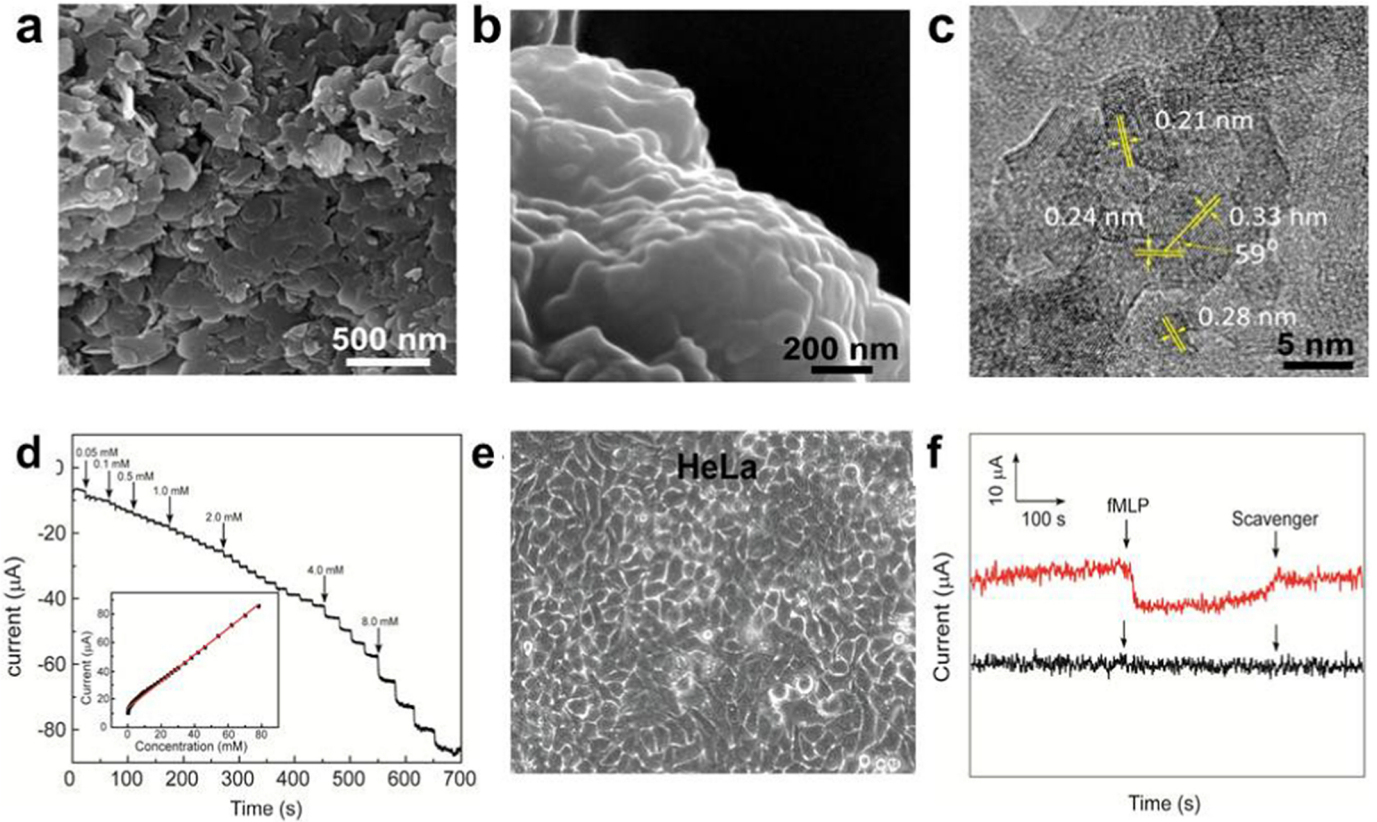
a
500 nm
b
200 nm
c
0.21 nm
0.24 nm
0.33 nm
59°
0.28 nm
5 nm
d
0.05 mM
0.1 mM
0.5 mM
1.0 mM
2.0 mM
4.0 mM
8.0 mM
current (μA)
Time (s)
Current (μA)
Concentration (mM)
e
HeLa
f
10 μA
100 s
fMLP
Scavenger
Current (μA)
Time (s)

Figure 8

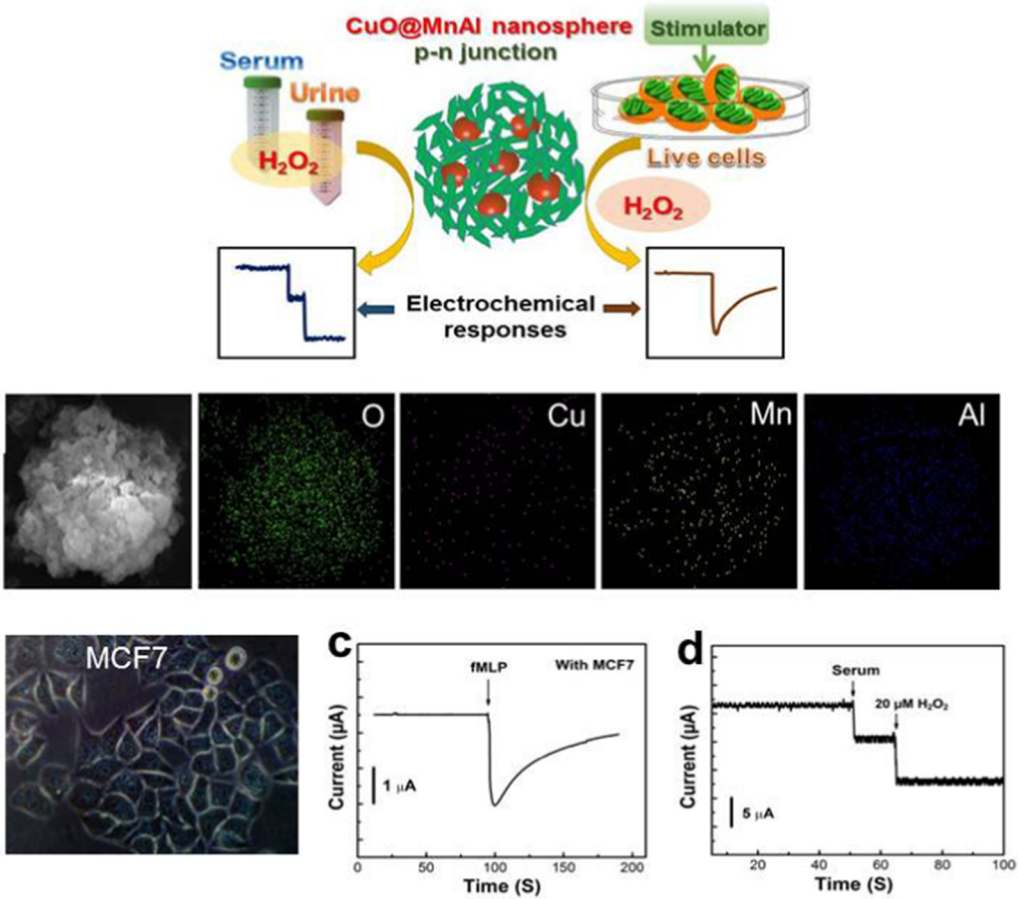
CuO@MnAl nanosphere
p-n junction
Stimulator
Serum
Urine
H_2O_2
Live cells
H_2O_2
Electrochemical responses
a
O
Cu
Mn
Al
b
MCF7
c
fMLP
With MCF7
Current (μA)
1 μA
Time (S)
d
Serum
20 μM H_2O_2
Current (μA)
5 μA
Time (S)

Figure 9

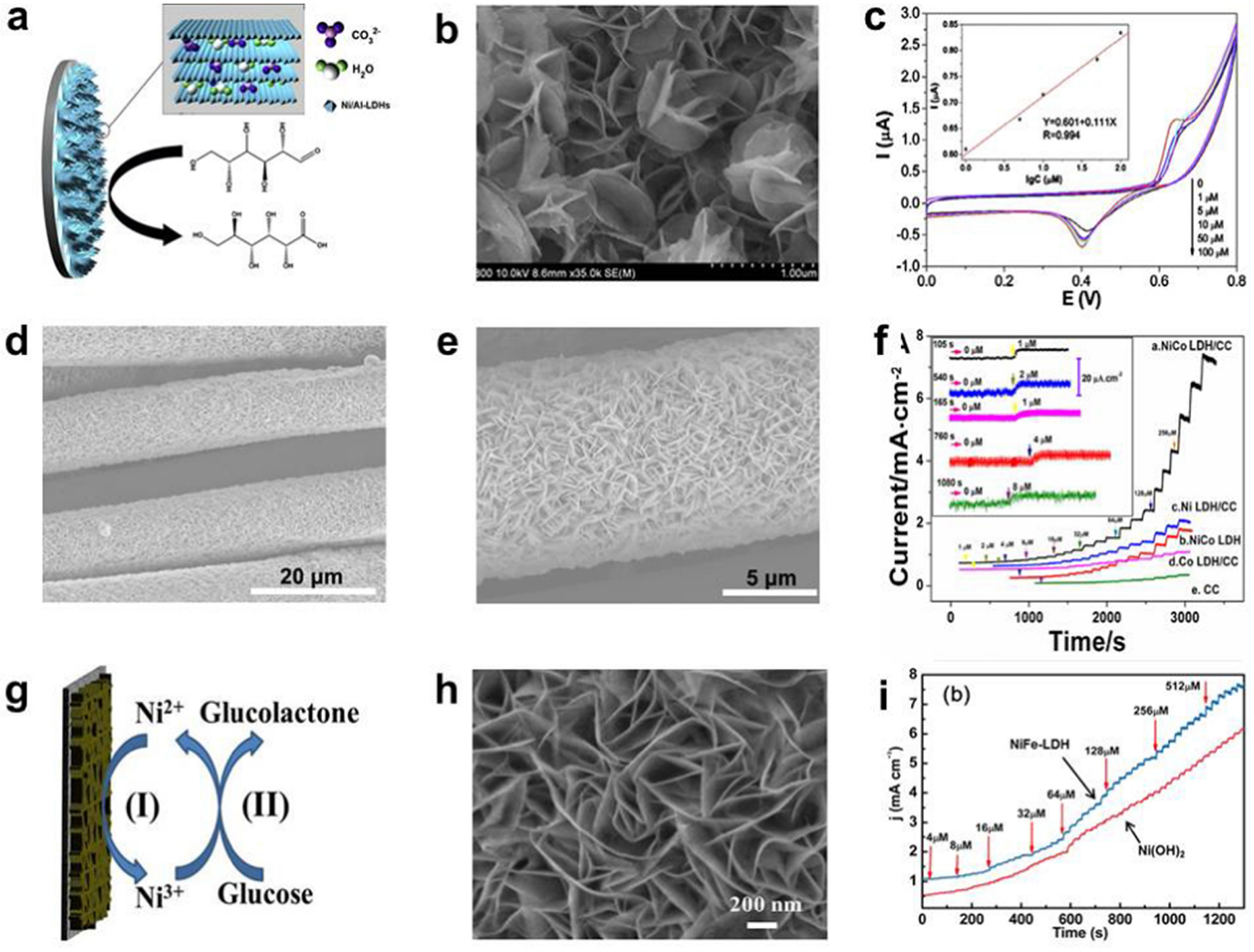
a
CO_3^{2-}
H_2O
Ni/Al-LDHs
b
c
I (μA)
E (V)
Y=0.601+0.111X
R=0.994
lgC (μM)
0
1 μM
5 μM
10 μM
50 μM
100 μM
d
20 μm
e
5 μm
f
Current/mA·cm⁻²
Time/s
a.NiCo LDH/CC
b.NiCo LDH
c.Ni LDH/CC
d.Co LDH/CC
e. CC
20 μA.cm⁻²
g
Ni^{2+} Glucolactone
(I)
(II)
Ni^{3+} Glucose
h
200 nm
i
(b)
NiFe-LDH
$Ni(OH)_2$
4μM
8μM
16μM
32μM
64μM
128μM
256μM
512μM
j (mA cm⁻²)
Time (s)

Figure 10

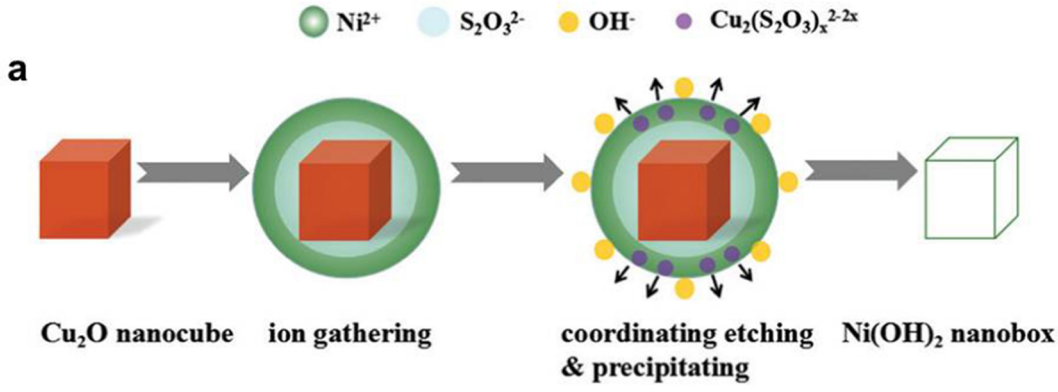
Ni2+
S2O32-
OH-
Cu2(S2O3)x2-2x
a
Cu2O nanocube
ion gathering
coordinating etching & precipitating
Ni(OH)2 nanobox
b
500 nm
c
50 nm
d
0.2 mM
0.5 μM
0.05 μM
0.05 mM
5 μM
I / μA
t / s

Figure 11