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Review Article

ROLE OF NANOMATERIALS IN THE DEVELOPMENT OF BIOSENSORS

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ABSTRACT
As nanotechnology is growing at a very high pace there is a need to reconstruct our concept regarding the tools (biosensors, chemical sensors) being used and envisage the possibilities to ensure their compatibility to meet the current challenges arising in the present scenario. Various materials used in the development of biosensors and the techniques involved have been rescreened based on interdisciplinary approach. Mostly, laboratory analysis by conventional modes of assays is becoming expensive and time consuming day by day. This is leading to the ever growing need for real-time, low cost technology which can be employed in industry, monitoring environmental and clinical and food processing. The concept of biosensors, transducers and receptors has been considered in order to find and fill the lacunae to be enriched with appropriate improved and modified versions to meet the challenges. The role of nanomaterials like metal, metal oxide, nanowires, nanocomposites, quantum dots, dendrimers and others has been discussed. The various receptors biological and chemical, both related to the sensors have been considered because biosensing is basically dependent on the molecular recognition; this is apparently a prerequisite of transducer to attain efficient workability. Receptors are the biological molecules which have the ability to receive and deliver a specific molecular, functional and detectable message.

KEY WORDS: Biosensors, receptors, transducers, nanomaterials, nanotechnology, cells and tissues, biomolecules.

INTRODUCTION

There is an immense thrust and intense interest in the field of development of biosensors to facilitate effective, quick, reliable, reproducible results of the analysis accomplished with high degree of sensitivity, accuracy, using gadgets easy to handle even by semi or non-technical individuals. There have been constant efforts in the field of biosensors technology with a focus to implement various nanomaterials either into transducer or components of bioreceptros to attain multi detection potential and sensitivity. The nanomaterials of interest like nanoparticles, nanotubes, quantum dots, dendrimers and other biological materials; as a result of the intense investigations the nanosized nanosensors, nano-products have brought revolution in biological and chemical analysis of many products *in vivo* (Sagadevan and Periaswamy, 2014). There are reports regarding varied innovative efforts in the sphere of sensing receptors, transducer and the most deserving support from electronic, soft ware and micro-fluids, all these facilitating the detection of multi-analytes instead of single analyte. Miniaturizing is playing a prominent role in the development of the devices which are focused to be highly sensitive, efficient, easy to be handled, ability to analyze large numbers of samples, provide reproducible results that too in short time and to the tune of fg/ml concentration of analyte/analytes (Tothill, 2011). In the recent past biosensors have become rapidly proliferating field with around 60% annual growth rate and prime impetus is from health care, personal care, food industry (food quality appraisal), environmental monitoring *etc*., Atta *et al*., 2011). Biosensors are amongst the most sought after analytic devices exploited in almost any aspect of industry encompassing food, health care, personal care, environmental care, electrical and electronics. Mostly, laboratory analysis by conventional modes of assays is becoming expensive and time consuming day by day. This is leading to the ever growing need for real-time, low cost technology which can be employed in industry, monitoring environmental and clinical and food processing. Thus, an entrepreneur is looking for miniaturization, integral systems and multianalytes determination to meet the current situation; this has diverted the efforts towards the development of suitable biosensors (Marazuela and Moreno-Bondi, 2002). Quality food and its safety is scientific discipline and it describes handling, preparation, storage and transportation of food in an appropriate way which prevents the food-borne pathogens and related illness; food is a favorable growth medium for the microorganisms, this growth may results in its spoilage and renders it non-consumable (Narsaiah *et al.*, 2012). Nanomaterials have been exploited to enhance the quality of food, its preservation, and efficacy of food technology and maintain the suitability and palatability; these parameters are the basis on which the selection of nanoparticles is made and the engineered devices are employed to check the quality, handling, storage and transportation of food (Lahir, 2015). Nanomaterials because of their specific physicochemical qualities and interactions are being considered favorably for fabrication of biosensors. Some of their qualities are strong catalytic activity, stability and least fouling to surface, thus, nanomaterials like carbon nanotubes, nanomagnetic beads, nanocomposites *etc*. are being considered for the

fabrication of nanobiosensors (Li *et al*., 2014). Electrochemically fabricated devices have many advantages like preparation of excessive sampling can be avoided, this makes the detection relatively easy; relatively more numbers of analytes can be detected without separating them from the biological sample; this is possible because specific signals are picked up and these signals are related to a particular biomolecule having characteristic potential; very small biological samples are required for detection of analytes (Sanghavi *et al*., 2014). Further, other advantages related to this technique include, relatively fast sampling, provides metabolic fate of the analytes, drugs, their inter action, dosages (if required). There are some limitations of this technique that include knowledge of the electrochemical or electro activity of the analytes, complexities due to interfering species which may be present in higher concentration as compared to the analytes under investigation, electrode fouling and charges may result in back ground drift and lack of strategy to carry out the specific detection. In this presentation an effort is made to review the current status of nanomaterials with respect to bioreceptros and development of nanobiosensors, their role and feasibility at laboratory level, commercial and industrial level. Thus, biosensors come to our rescues and are associated with micro-electric technology.

BIOSENSOR & THE BASIC CONCEPT BIOSENSOR

Biosensors are the specialized analytical devices capable of converting a biological response into a detectable electrical or electronic signal. A given biological response is mostly expressed in relation to the concerned enzyme, over all cell metabolism, ligand-binding, antibody-antigen interaction etc. The field of development of biosensors is very vast and involves the basic theoretical and practical concepts from biochemistry, physical chemistry, bioreactor science, electrochemistry, electronics and soft-ware engineering skills. These fundamentals facilitate the fabrication of biosensors to be very precise and effective analytical tool to meet the changes of modern challenges. All these analytical innovations are dependent on the central dogma related to the molecular recognition, its behavior and ability of biosensor to detect the biological response (Martin, 2012).

It is very essential for a prompt analysis that biocatalyst should be stable under normal/routine working conditions and be stored. This stability should withstand large numbers of assays, say more than 100. This feature is not applicable to strips and dipsticks used in colorimetric enzyme device. The reaction involved in the related device should not be affected by the physical parameters like stirring, pH and temperature etc., to minimize pretreatment during spot analyses. In general, enzyme reactions involve coenzymes and/or cofactors; in such cases, it is very essential to immobilize the coenzymes and cofactors along with the related enzyme under investigation. One should make sure that the respective response should be very accurate, precise, and linear over a convenient detectable range without dilution or concentration and should be reproducible. Further, as far as possible, it should be free from electrical noise. There is a possibility that this inherent noise of electric and/or electronic device is likely to make the detection of the specific signal (response) difficult or interfere with the detection because of these unwanted random signals may be picked up during the process of detection of biological response in terms of electrical signal (Atta *et al*., 2011; Martin, 2012). Biosensors are used specifically in case of invasive monitoring under clinical situations, the probe used should be tiny, biocompatible and either non-toxic or non-antigenic and non-causative agent. The probe used may vary for different processes like fermentation, synthesis etc. In case of fermentation, the probe should be able to withstand the parameters of sterilization i.e. autoclaving. Further, care is to be taken to prevent fouling or proteolysis. Some of the promising qualities of biosensor are cost-effectiveness, small size, portable and easy operation even by semiskilled technician; preferably no specific training should be required for its operation. Proposed fabricated biosensor must have appropriate market and should be consumer friendly. It should accomplish decentralization of laboratory analysis and in all probabilities it should be acceptable in the specific industry and also by the consumer.

WORKING OF BIOSENSORS

Basic concept of working of biosensors involves the conversion of reactant into a product. This is accomplished by a biocatalytic membrane; in turn biocatalytic membrane determines the biological response of a given biosensor. Working of biosensors at different levels/stages is accomplished in a stepwise pattern, binding of bio-receptor with specific form of sample, formation of electrochemical interface at the sight or step of occurrence of biological process/processes resulting in the signal thus, a specific biochemical reaction is converted into a specific electrical signal involving transducer; conversion of electronic signal into a readable/meaningful physical parameter to display the respective results to the operator (Sagadevan & Periaswamy, 2014). Biocatalytic membranes are biohybrid artificial systems in which biochemical conversion takes place along with membrane separation process. In this case chemical conversion is catalyzed by a catalyst of biological origin. The membrane bioreactors are tools for the improvement of traditional system to maintain sustainability as these are cost effective, need less space, low energy consumption, compact and flexible *etc*., (Giorno and Drioli, 2002; Giorno and Drioli, 2010). The ability of an enzyme to get immobilized is a unique intrinsic and/or extrinsic property that helps to stabilize the enzyme system. Immobilized enzymes elevate the resistance towards factors like pH, temperature, immobilized enzyme held in place throughout the reaction and at the same time it is isolated from the product. This phenomenon plays significant role in reducing allergic reactions, hence, immobilization of enzyme is of chemical, molecular and economical benefits (Zaushitsyana *et al*., 2014; Aragao *et al*., 2014). The ability of reusability of immobilized enzyme is responsible for securing catalytic activity for series of analyses. As a result of this, the catalytic redundancy, *i.e*., provision of multiple interchangeable components to perform a single function in order to provide and enhance the degree of stabilization of the immobilized enzyme/s under investigation. Quite often immobilized enzyme undergoes

some inactivation over a period of time, this inactivation is predictable and steady in nature. If at all decline/decay in activity takes place, it can be easily incorporated into an analytic process. This is achieved by interpolating standard between the analyses of unknown samples. Such feasible and workable options make many immobilized enzyme systems reusable up to 10000 times over period of many months. Such features bring in cost effectiveness and efficient analytical usages of free soluble enzymes. Kinetics of external and internal diffusion is another aspect that is considered because it affects the enzymatic reaction (Mohaghehi *et al*., 2007). The rate of external diffusion influences the reaction in which immobilization of an enzyme occurs along with the membrane of a biosensor, this factor reflects on the proportional fluctuations in the rate of reaction with respect to substrate concentration, linear range related to intrinsic (K_m). This factor is the basis of using concentrated enzymes without dilution during the analysis in comparison with free enzyme in solution (Martin, 2012). Parameters like pH, ionic strength, inhibitors etc, related to the said enzymatic reaction can also be investigated. Further, the problems which arise because of variability of real analytical samples along with external conditions when fermentation broth, blood and urine are investigated can be easily resolved. When one uses permeable membrane between an analyte enzyme and bulk solution, the external diffusion of analyte can be easily and effectively enhanced. This technique is quite useful to control the response and the working of biosensor. The thickness of such permeable membranes can be different and is associated with the proportionality constant; this in turn is related to the concentration of substrate and the respective rate of reaction. For example, when there is an increase in the thickness of the membrane, it increases the unstirred layer; this declines the proportionality constant. If total dependence on external diffusion rate is not attained or becomes attainable and if any enhancement takes place in dependence of reaction rate related to either external or internal diffusion, it reduces the dependence on pH, ionic strength, temperature and concentration of inhibition.

Over all, around three generations of biosensors have been in the service of mankind (Atta *et al*., 2011; Li *et al*., 2014; Martin, 2012). First generation of biosensors involves diffusion of normal products to the transducer and it results in electrical response. In case of second generation of biosensors there is a development of "mediator" between the reaction and transducer, this enhances the quality of response. The basic working of third generation of biosensors is concerned with release of response as a result of reaction and diffusion of either specific product or a mediator. The general working principle is based on the fact that the biological response is in the form of an electrical signal which is released from transducer. It is quite often low and is superimposed upon relatively higher and noisy base line. This means the electric signal (response) contains high frequency signal component, its nature is either random because of interference of electrical nature or produced by the electric component of transducer. Under normal conditions the signal processing, "reference" base line signal is subtracted from a similar transducer having no biocatalytic membrane from the sample signal and amplifying the resultant signal. The purpose of this exercise is to filter *i.e.* smoothen the unwanted signal noise. The responses due to biosensor are relatively slow in nature and it can ease the problems related to the filtration of electrical discrepancies. At this step similar signal is produced and output is directly received. Usually the signal is changed into a visual signal and conveyed to microprocessor - a site of conversion or processing and the electrical signals are converted into concentration units. This data is either displayed or stored.

WORKING OF TRANSDUCER

The physical changes which accompany the reaction related to the working of transducer of biosensors include production or absorption of heat during the reaction that can be investigated by calorimetric biosensor (Se Chul *et al*., 2007); a change can take place in the distribution of charges that can produce electric potential, it is analyzed by potentiometric biosensor (Psychoyios *et al*., 2013). There can be a movement of electrons (e^-), these electrons are produced during redox reaction; this is investigated by amperometric biosensor (Liu *et al*., 2005). There are chances that either light can be produce or light can be absorbed during the said reaction between the reactants and/or products which is detected by optical biosensors Cush *et al*., 1993); during such reactions there can be some fluctuations due to the mass of the reactants and products, these are studied by piezoelectric biosensors (Gautschi, 2002; Moubarak, 2012).

RECEPTORS

Biosensing is basically dependent on the molecular recognition and is apparently a prerequisite of transducer to attain efficient workability. Receptors are the biological molecules which have the ability to receive and deliver a specific molecular, functional and detectable message. The biomolecules which play a key role in biosensing are primarily the product or byproducts of specific physiological/ biochemical/metabolic processes/pathways; these are also natural biochemical target. These also appear to be "workable" targets for xenobiotic, toxic and nontoxic (Lahir, 2012) and for biosensing (Shaikh and Patil, 2012). Receptors are related and associated to plasma and intracellular membrane, these exhibits specific binding to the corresponding specific micro and/or macro molecules and these are referred as ligand. The innate behavior of this association indicates the 'response' which may be cellular, biochemical or physiological in nature and function. The mechanism of the interaction between ligand and receptor is likely to involve conformational, structural changes which either involve or favor association with the components of biological membrane such as opening of channels, activation or generation of second messenger mediated by adenyl /guanyl cyclase and other transductive responses. These responses involve G proteins, enzymes like tyrosine kinase, phosphatase, phosphorylase etc; factors affecting transcription, antigen involving cell receptors (Shaikh and Patil, 2012). The process of recognition of biomolecules is typically and exclusively specific, reversible, exhibits affinity and can be generalized to an affectively unlimited range involving aqueous analytes. These features are recognized and widely exploited as diagnostic and synthetic tools

(Mohammed, 2010). Electrochemical interaction is based on charges, dipoles and quadrupoles; these interactions may be accomplished in number of ways such as the ability due to the interacting components causing induction and dispersion forces which cause polarization of charge distribution inter-atomic repulsion related to Pauli-exclusion (Kahn and Plaxco, 2010; Massimi, 2005). Aqueous ambient environment declines the impact of electrostatic and induction interaction to a greater extent, thus, mostly hydrophobic effect becomes a major force which plays a key role in stabilizing the appropriate biomolecules-target complex formed. Destabilization of chemical binding within the biomolecules and/or interacting partners including target also have their role in recognition of biomolecules or receptor (Narsaiah *et al*., 2012). Receptors like enzymes, whole cells, nucleic acids, antibodies and biomimetic polymers have been used during the process of biorecognition (Marazuela and Moreno-Bondi, 2002). Range of analytes formed due to pathogen or during storage, in food samples have been analyzed (Narsaiah *et al*., 2012). Role of nanomaterials is very useful for the molecular diagnosis (Bapista *et al*., 2011).

ENZYMES

The receptors can also be called bio-component and biomolecules like enzymes, antibody, nucleic acid, lectin, hormone, cell structure or tissue can be used for the detection of deviation from normal pathway or any structural biochemical dysfunction (Monosik, $2012_{1\text{-}a}$). Enzymes have specificity, selectivity and efficiency; other aspects of enzymes of consideration are their affinity for the desired molecule and catalyze the formation of product that product can be directly analyzed using corresponding transducer. Based on this parameters it may be suggested that categories of enzymes with specific functions and uses for their competent substrate and the product formed becomes the bases for the detection by the corresponding transducer (Monosik, $2012_{1\text{-}a}$). Some of such categories of the enzymes and their functions which have been involved in the development of biosensors include oxidoreductase-regulating oxidation/reduction reactions; transferases-bring about transfer of molecular group from one molecule to other; hydrolases cause hydrolytic cleavage; lyases-cleave the bonds like C- C, C- N, C- O by other mode than the oxidation or hydrolysis; isomerases-bring about the rearrangement within the molecule and ligase join two molecules. One of the most successful and commercially available biosensor is for detection of glucose in blood and it involves glucose oxidase or glucose dehydrogenase enzyme; this biosensor represents around 90% of the global biosensor market, this is in relation to the diabetic individuals all over the world (Martin, 2015; Monosik, $2012_{1\text{-}a}$). Enzymes are very suitable analytic reagents; the rate of such reactions can be estimated from the difference in the optical absorbance between the reactants and the products. This can be illustrated by the example of β-D-galactose dehydrogenase assay for galactose in which oxidation of galactose is accomplished by redox coenzyme, nicotine-adenine dinucleotide (NAD^+); a 0.1mM solution of NADH shows 0.022 as absorbance at 340nm while NAD^+ shows zero absorbance at 340nm. This conversion involves large increase in light absorption at 340nm. When the concentration of galactose is kept below the K_m of the said enzyme involved then the reaction exhibited linear pattern (Martin, 2015). U-shaped biosensor has been developed for detection of blood glucose involving localized 'Surface Plasmon Resonance' based optic fiber (Shrivastva *et al*., 2012). Some of the other enzymes involved in the construction of biosensors are oxidative reductase enzymes used for detection of lactose (Huang *et al*., 2009); malate (Arif *et al*., 2002; Monosik *et al*., $2012_{\text{-c}}$); ascorbate (Vermeir *et al*., 2007; Wang *et al*., 2008); amino acids (Pollegioni *et al*., 2007); alcohol (Smutok *et al*., 2006); cholesterol (Ahmed *et al*., 2013); glycerol (Monosik *et al*., $2012_{\text{-d}}$); fructose (Tkac *et al*., 2002); benzoic acid (Hairul *et al*., 2011). Transferase enzyme was involved in biosensor for detection of acetic acid (Mieliauski *et al*., 2006) and determination of xenobiotic like 'Captan' or atrazine was carried out using relative biosensors respectively (Choi *et al*., 2003; Andreou *et al*., 2002). Other enzymes studied were hydrolases in case of sucrose (Soldakin *et al*., 2008); lyases to detect citric acid ((Mieliauski *et al*., 2006), ligase in the detection of DNA point mutation (Pang *et al*., 2006) and isomerases to investigate 19-norandrostenedione (Sheu *et al*., 2008). Various factors which influence the working of enzyme based biosensors include loading of enzyme, pH, temperature, and the cofactor (if involved); these are associated with the retention of abilities of enzyme under investigation (Monisik *et al*., $2012_{\text{-1-a}}$). When enzyme immobilization technique is in use then electrode performance and the thickness of the layer of enzyme in/on biosensor also play significant role in the analytical function of a biosensor. During conducting enzyme assays some of the drawbacks are faced on a large scale when large number of estimations are to be conducted, these are seen in terms of cost of enzyme and coenzyme usage, time involved, need of skilled workers and reproducible results and operations etc, (Martin 2012). These are to overcome during routine or onsite assays.

ANTIBODIES

Antibodies are highly specific and specialized molecules, these features can be exploited in the field of development of biosensors. Antibody is a biomolecule made of amino acids that are arranged in a specialized and unique pattern hence their structural and functional specificity are the fundamental bases in the analytical technology and immunosensors. This is further elaborated by the specific product and/or reactant exhibiting specific binding sites between relevant antigen and antibody. Variable selectively antibodies are effective tools that can facilitate the detection of wide range of analytes having low molecular weight to words agrochemical like herbicides, insecticides etc, to complex proteins, structures of pathogenic microorganism (Kramer *et al*., 2008). Detection can be carried out up to nano-molar or even higher concentration without extensive pretreatment of the samples. Antibodies are the sources of either isolation or detection of immune-reagents for xenobiotic, pesticides, pathogens or genetically modified organisms. These can be rationally designed or directed according to the specific conditions; the molecular format can be modified to the directed immobilized or sensing surface or integration of

markers which can be manipulated or engineered as fusion protein. Stability is one of the weaker aspects of antibodies as compared to biomimetics *e.g.* molecularly imprinted polymers (MIRs). These can be engineered to enable antibodies to function under abnormal conditions like thermal pressure in presence of denaturing sample components. Recombinant antibodies are used as a new reagent for biological detection (Emanuel *et al*., 2000). Interaction between antigen-antibody may be grouped in to two categories; first category is referred as direct format and involves interaction between target molecule and ligand molecule, one of the two can be immobilized. The direct measurement can be done by using fluorescent analyte after appropriate incubation *in situ*. The second category is called indirect format, in this case separately labeled species (molecules) are bound with fluorescent dye or luminescent dye followed by their detection. In this case basically non-labeled analytes behave like competitors against labeled analyte having restricted number of binding sites for receptors (Monosik *et al*., $2012_{1\text{-}a}$). The bases of estimation are related to the degree/number of changes in the labeled signal that takes place during the formation of analyte-labeled conjugates *i.e*. immune-complex and antibody. The reactants are allowed to interact by mixing them with sample and their responses are measured kinetically. If the amount of immobilized reagent declines the degree of sensitivity of assay enhances (Monosik *et al*., $2012_{1\text{-}a}$). The antibody based recognition is accomplished involving optical, electrochemical signal transduction technique, enzyme based, and fluorescence based techniques; the degree of amplification and the signal resulted has enhanced the sensitivity of the assay (Chambers *et al*., 2008). Routine assay of Prostate specific antigen (PSA) using enzyme-linked immunosorbent assay (ELISA) involved the formation of specific PSA immune complex. Protein ligand interaction generates nano-mechanical motion on microcantilevers, it can lead to immobilization of PSA antibody and this element is useful in detection of PSA in serum. The probable explanation is that molecules adsorbed on a microcantilevers, caused changes in the vibrational frequency, these are referred as 'curling'-change in frequency is due to stress caused on one side of the cantilever as a result of adsorption. Noble metals affect 'Surface Plasmon Resonance' based optical transduction and this feature facilitates the determination of antibody. If such techniques are developed by micro-fabrication, these are probably being useful in the development of suitable biosensors that may enhance clinical or other determination (Chambers *et al*., 2008). Recombinant antibodies can be genetically manipulated (Emanuel *et al*., 2000). Many recombinant antibodies are useful to detect and identify diseases like HIV, Hepatitis B and C, Simian immunodeficiency, Ebola, Rabies, Epstein bar, measles virus and botulinum neurotoxin A/B ((Chambers *et al*., 2008; Petronko *et al*., 2004; Zeng *et al*., 2012). Combined innovative combined techniques of polymerase chain reaction (PCR) and nucleic acid detection have been developed to detect relevant tumor marker, viral proteins, toxins metabolites and pathogen. Even antibodies are being employed in sensing technique involving nontraditional immune-reagents *i.e*., 'ion channel switch' is one such technique based and is based on self assembling of synthetic biomembrane (Krishna *et al*., 2003). The biosensing element is made of two molecule layers; it is self assembled membrane structure similar to ion channel peptide germicidin. The analyte binds to antibody tied to the channel and changes the population of conduction ion channel paired within membrane (Krishna *et al*., 2003). Human diseases can be detected very fast by using inexpensive, noninvasive immunoassays. The quality, specificity and biological sources/origin of antibody are the parameters and are responsible for the complexities in the detection noninvasive immunoassay. These problems are overcome by adopting genetic engineering and recombinant antibody technique; generally formation of antibody takes more time in human and still more in other animals; with the help of recombinant antibody technique antibodies are formed in bacteria, yeast or other types of cells (Zhang and Ning, 2012). Under normal traditional enzyme-linked immunosorbent assay (ELISA) mode of formation of antibodies requires two or more antibodies or fragment of antibody to find the specific antigen although it is time consuming, costly and represent a specific disease; these adversities are solved by adopting recombinant antibody technique. The recombinant antibodies can be engineered genetically to a self assembled surface of biosensor at high density; further, these can get suitably self oriented to facilitate and increase the degree of antigen-binding ability which plays a key role in the assay, it also enhances sensitivity, specificity and stability (Krishna et al, 2003; Zhang and Ning, 2012). The physical, electrical and chemical properties of the biosensors surface can be oriented in order to elevate the degree of performance of immunoassay in comparison to that of traditional method.

NUCLEIC ACIDS

Nucleic acids are the macromolecules primarily concerned with genetics and protein synthesis; these are employed in the field of clinical analytical, genotoxic, antibodies identification and detection. Biosensors having the ability to interact with nucleic acids are called genosensors. The basic principle of the interaction is based on the quality to recognize precisely the complementary base pairs (ATGC) in a given nucleic acid. When the sequence of base pairs of nucleotides in target nucleic acid is known, then complementary sequence can be fabricated or synthesized and labeled, this is followed by immobilization on a specific sensor. Technically in the molecular field hybridization probe is considered to be a fragment of DNA or RNA having the length containing 100 to 1000 base pairs and used to detect the nucleotide in the target DNA/RNA which is complementary to the sequence in the probe hybrid. The labeled probe is treated with NaOH (alkaline) or thermally, to denature it and single stranded DNA is formed. Thereafter, it is hybridized to DNA involving Southern blotting technique or RNA involving Northern blotting technique followed by their immobilization, (it may be done in situ also) (Strachen *et al*., 1999). DNA point mutation is based on the DNA ligase reaction and is detected using piezoelectric approach (Pang *et al*., 2006). Hybridization probes are allowed to base pair with the target sequence involving the generation of an optical signal. DNA biosensors are useful

tool in clinical diagnostics, agriculture, forensic science, and environmental monitoring and clean-up efforts.

Basically a DNA biosensor is made of sensing elements, micro laser and generator of a signal. Basically the sensor exactly fits *i.e.,* the strands of DNA matches up with the every nucleotide position or matching a fluorescent/glow as a signal is released, this signal is transmitted to the generator that converts it in to readable or recordable signal. High sensitivity and selectivity is derived from the base pair affinity between complementary nucleotides present in the region under investigation (Borgman *et al*., 2011). Natural and biomimetic forms of oligo and/or polynucleotide function as recognition element is involved with respect to the genobiosensor (Monsik, $2012_{1\text{-a}}$). Recently oligo-oxyribonucleotides have been synthesized and used as probe in DNA hybridization sensors; terminal components/groups like thiol, disulfides, amines or biotin have been incorporated with immobilized oligo-oxyribonucleotides to the surface of transducer (Labuda *et al*., 2010). A specific longer flexible spacer has been used by providing hydrocarbon linker in order to enhance the accessibility to wards surface attachment. Aptamers are selected randomly from oligonucleotide library using 'systematic evolution of ligands by exponential enrichment' (SELEX).

The aptamers used are very efficient and various non-nucleic acid targets such as proteins, cells, viruses, bacteria, other smaller molecules like dyes, metal ions and amino acids can be detected. The aptamers are quite similar to monoclonal antibodies and have specific binding affinity, relatively more resistant and can withstand greater degree of degradation and/or denaturation. Further, their abilities related to affinity and specificities can be designed rationally by using techniques of molecular evolution. Protein is detected by using aptamer biosensor (Stoltenberg *et al*., 2008); DNA aptamer derived from capture-SELEX procedure has been used to detect a relatively small molecule target, aminoglycoside antibiotic kanamycin-A. Immobilized nucleic acid aptamer is feasible 'flexible biosensor' tool to detect free non-labeled non-nucleic acid targets like protein (Stoltenberg *et al*., 2012). Detection of antithrombin has been carried out by labeling antithrombin aptamer with fluorescein; fluctuations induced in fluorescence anisotropy of the immobilized aptamers are used to detection thrombin in a sample solution (Potyrailo *et al*., 1998). More stable and suitable aptamers are being developed to meet the requirements for fast and easy detection of non-nucleic acid target like protein. Aptamers exhibit specific and higher affinity for non-nucleic acid target moieties; the binding interactions make them suitable for biorecognition element for biosensor/genosensors and their determination (Nikolaus and Strehlitze, 2014). For detection of antibiotic residue against antibiotic resistant pathogen, the aptamers should be adjusted to meet the specific affinity requirements. The pattern of binding between aptamer and specific related amino glycoside antibody can be estimated within micromolecular range. Aptamers developed are highly versatile in their efficacy; this is further elevated when aptamers are associated with nanosystems. This association elevates the range of applications like imaging, drug delivery and other medical applications. Target affinity can guide /direct the transport of aptamer-nanoparticle conjugate to its site (if interact) and it can also improve cancer related parameters (Reinmann and Strehlitze, 2014). The immobilization technique has been applied to DNA-probe and it has enhanced the performance of biosensors/ genosensors (Monsik, $2012_{1\text{-a}}$). Some of the reports as on the use of materials like carbon paste (Girousi *et al*., 2014); pyrolytic graphite (Chen *et al*., 2010); glassy carbon (Pedano and Rivas, 2003); carbon fibers (Tian *et al*., 2005); carbon nanotubes (Mani *et al*., 2009) are available. Genobiosensor or DNA dependant biosensors have been employed successfully in the detection of drugs in blood serum ((Girousi *et al*., 2014; Vanikova *et al*., 2005). DNA Damage and antioxidants which protect the DNA ((Labuda *et al*., 2010; Galandova *et al*., 2005; Vyskocil *et al*., 2010); determination of effects of berberine released from cancer cells on DNA (Ovadekova *et al*., 2006) are used in the field of functioning of DNA dependant biosensors or genobiosensor.

EPIGENETIC MOLECULES

Epigenetic is a study of the changes in the expression of genes either due to "turning off" or "turning on" of some of the base pairs via chemical interactions. Mostly such conditions arise during DNA-methylation and/or modifications of histones. During this transformation the sequences of nucleotides do not change; at times, when 'silencer proteins' are active either change in sequence of nucleotides or expression of genes do not occur. Cellular functions like differentiation, morphogenesis, change of totipotent stem cells in to pluripotent cell-line of the embryo, are accomplished during epigenetic gene regulation. In addition to these activities other activities such as formation of epithelium, neurons, muscles cells, endothelium etc, also take place during epigenetic regulation. Further, activation of some genes occurs while inhibition of expression of other genes can also take place during epigenetic genetic regulation (Reik, 2007). Methylation of mRNA and demethylation of N6-methyladinosine in RNA can affect the epigenetic gene regulation (He, 2010; Guifang *et al*., 2011).

Some disorders in human are related to the process of imprinting of genes; during this process father and mother provide different epigenetic pattern for specific loci in their gametes and due to the presence of genomic imprinting, a specific region in a given chromosomes is affected (Knoll *et al*., 1989; Wood and Oakey, 2006). DNA-methylation can cause cancer; some germ line mutations are possible and these mutations are responsible for at least 2%-5% of colon cancer (Jasperson *et al*., 2010). Aberrant DNA is responsible for unscheduled gene silencing while 5-methylcytosine can bring about transcriptional silencing of those genes in which concentration of 5-methylcytosine is high; hyper-methylation is one of the major causes of repressing genes during transcription (Wong and Craig, 2011). This factor is responsible for different mechanisms for development and progress of cancer. Presence of cell free fetal (cff) DNA and RNA in the maternal blood were detected by noninvasive prenatal diagnosis. As a result, the cff-DNA and/or RNA have become the prime targets of investigation (Puszyk *et al*., 2007). This aspect has evolved into new strategies to ensure the distinction and

quantitative assay in the maternal blood samples of these cells free fetal DNA and RNA.

The process of interaction between epigenetic molecules, biomolecules and the respective detector molecules is accomplished because of bimolecular recognition (Mohammed 2010). Further, this interaction is related to surface recognition by receptors preferably those which are immobilized. Recognition of receptors is fabricated in an appropriate pattern so that these molecules are able to mimic the naturally occurring epigenetic and other concerned molecules that function as analyte. These innovations when act in a coordinated manner facilitates detection by biosensors, microarray and analyte assay. Such molecules include engineered antibodies, carbohydrates, nucleic acids, oligonucleotides, aptamers, genetically engineered cell proteins, phage display, molecularly imprinted polymers and biomimetic materials. Some of these functions as elements of molecular recognition (Mohammed, 2010).

Optical biosensors are advantageous because they help to relate various targets desired for detection in original form along with features like easy handling, portability, lesser volumes of test samples *etc*. These biosensors are good tools to detect modified histones, post-translational and epigenetic markers (Donzella and Crea, 2011). Biomarkers related to ovarian cancer namely fibronectin, apolipoprotein A-1 and TIMP-3 (tissue inhibitor metalloproteinase -3) have been detected by employing quantitative immune-blot and 'guided mode resonance' (GMR) technique using label free optic biosensor read out (Kaja *et al*., 2012). These markers have potential for the differential diagnosis for the varied stages of metastatic ovarian carcinoma. These techniques for the detection of the relevant biomarkers were accurate, consistent and provide reproducible results which otherwise were not as per the requirements. Various approaches have been explored to investigate the increase in the degree of interaction between light and the desired analyte by matching the architectural characteristics and investigated 'transverse magnetic (TM) and transverse electric (TE) disc and ring resonators. TM device with higher potential for sensitivity and better capabilities to sense when penetrating distance into the analyte is increased (Ford *et al*., 2013). 'Stot wave guide Bragg grating sensors' were used in this study and exhibited enhanced sensitivity. Ultimately binding abilities were compared; architectural aspects were validated to attain higher level of suitability for biological and clinical applications.

Multiplexed label free biosensors involving nanostructured gold-polymers surfaces are provided with Surface Plasmon Resonance mode and this could be explored by a miniaturized optical setup. The chip used in the sensing device was responsive to sensitivity, detection limits and changes in refractive index (Battazi *et al*., 2014). A multiplexed label-free biosensing device having characteristic degree of sensitivity and lateral resolution are developed for a biological model. It could detect the inflammatory markers too (Battazi *et al*., 2014).

CELL ORGANELLES

Cell organelles are the structural and functional compartments present within the cell, these carry out specific biochemical pathways which in turn accomplish cell physiology in totality. The organelles acquire or synthesize almost all the required metabolites, receive specific signals and also release the specific metabolites as per the need of the type of the cells for its normal functioning. This exercise in totality brings about co-ordination, homeostasis between the cell and its ambient environment including extracellular matrix (Lahir, 2015). The particular metabolite of the related organelle can act as a receptor and give response to a biosensor. Mitochondria have attracted relatively more attention of researchers; it plays significant role in Ca^{++} homeostasis during physiological functioning, controls the organelle and complexities of Ca^{++} signaling in addition to the formation of ATP (Rizzulo *et al*., 1990). There appears to be every possibility of having a potential to reciprocate the functioning of Ca^{++} channel during stress or exposure to abnormal metabolites. The mitochondria play a regulatory role in the checking pollution due to detergents in aquatic media (Bragadin *et al*., 2001). Cell organelle like cell membrane, mitochondria, vacuoles can be a suitable test material to assay nutritional, oxygen delivery or supply, as these exhibit fluctuations during osmosis and pH fluctuations in there ambient medium.

Lysosomes have also attracted the attention of researchers; the membrane of lysosome facilitates the fusion of B-Cell-receptor carrying endosomes to lysosomes. The cross-linking of B-Cell-receptor brings about a change in the pH of lysosome and exhibit condensation of chromatin on exposure to phosphotidylserine (He *et al*., 2005). This feature reflects on the receptive behavior or nature of the lysosome membrane and the lysosome it-self. The internalization of peptide motifs which get localized on different intracellular compartments, get chemically fused with penetratin. Internalized receptor-independently get associated with mitochondria resulting in its death (Rangel *et al*., 2012). This observation potentiates cell biology with respect to the development and delivery of drugs. The surface of the membrane exhibits modifications during nutrition, defense, and resistance against stress, host immune system and during adaptation to adverse surroundings. Membrane vesicle formation is of common occurrence on cell surface, bacteria, archaea, fungi and parasites; this influences the physiology of pathogenesis. The functionalities of membrane are likely to be more pronounced during delivery of enzymes, toxins, communication signal etc (Deatherage *et al*., 2012). There is every possibility of membrane vesicle exhibiting some degree of response to the mediated functional features (Deatherage *et al*., 2012).

CELL AND TISSUES

Cells and tissues are living entities and exhibit sensitivity with respect to their internal as well as external ambient environments by giving appropriate responses to the respective stimuli. Cells show a tendency to get attached to the surface, remain alive for longer duration as compared to their organelles and most of them are capable of proliferation. Since, tissue is a collection of cells and the extracellular matrix in which the respective cells are retained; both of these components of tissue demonstrate features which function in coordinated and cooperative manner (Lahir, 2015). The overall numbers of cells more or less constant and respond to the various stimuli and

sustain in the respective functional physiological state (Lahir, 2015). As a consequence of the coordinated and cooperative functional aspect cells and tissues are very suitable units as preferred bio-receptors. These bio-receptors can be used to monitoring metabolic fluctuations, stress, xenobiotic, inorganic and organic derivatives (Lahir, 2012); temperature, pH, ionic concentration, pressure etc, (Monosik, $2012_{1\text{-}a}$). Although microbes, fungi, animal and plant cells and tissues exhibit basic differences but their responses to the stimuli are the reflection of their degree of sustainability. These become the fundamental basics of monitoring techniques. Biorecognition plays key role with respect to cells or tissues microbes, and/or specific binding capacity of biomolecules; these molecules are referred as bio-receptors (Monosik, $2012_{1\text{-}a}$). There is a need to fabricate suitable biosensors to detect the bioreceptros that are likely to be present in very low concentration. For instance microbial biosensors are utilized and involving viable and nonviable microbes to detect any bio-receptors. For different molecular bio-receptors, viable and nonviable cells are used depending on the analyte to be detected.

MATERIALS FOR BIOSENSORS

Biosensors and transducers work in coordination and both are fabricated in accordance with quality, mode of interaction and specificity of "in-put" and "out-put" aspects of biosensors. Transducers can be grouped as electrochemical, electrical, optical, piezoelectric and thermometric; accordingly biosensors are categorized as amperometric, potentiometric, conductometric, ion-sensitive, acoustic and microcantilevers (mass-sensitive), calorimetric etc, (Monosik, $2012_{1\text{-}a}$; Reza *et al*., 2013; San and Rogach, 2010). The major thrust in the field of development of nanobiosensors is to facilitate effective, quick, reliable, ability to give reproducible results of the assay; these devices are highly sensitive, accurate, easy to handle and also able to analyzed large numbers of samples 'on sight'. Miniaturization is the prime factor that contributes to carry out detection in less than the duration of a minute and concentration up to fg/ml, various types of materials are being experimented in order to evolve a biosensor which works to the expectations related to time, efficiency, handling and accuracy. Metals, non-metals composite materials have been exploited to meet such expectations and efforts are being made to improvise even synthetic materials Reza *et al*., 2013.

METALS AND METAL OXIDES

Noble metals crystals and metals like gold, silver, palladium, platinum and/or rhodium in pure, alloy and/or core/shell forms are quite suitable material to develop a biosensor by using aqueous and organic techniques San and Rogach, 2010). Wide variety of shapes have been involved in the fabrication of biosensors like sphere, rod, cube, triangular, hexagonal nanoparticles; metals like Au, Zn and their oxides are also incorporated in designing the biosensors (Yuan and Wang, 2010; Narayanan, 2012). The applications of biosensors are based on specific instrumental "read out" methods like surface enhanced 'Raman spectroscopy', 'Surface Plasmon resonance' and 'electrochemistry'. Specific bioanalytes have been detected because of the unique properties of noble metals nanoparticles. The physicochemical properties include the ease of functionalization involving simple basics of chemistry, high surface to volume ratio, spectral and optical properties. The nanoparticles of noble metals also facilitate the techniques involved in fluorescence, infra red and Raman spectroscopy. Further, these nanomaterials play a key role in the process dealing with the synthesis, functionalization and integration with biomolecular diagnostics. The biosensors developed from noble nanoparticles are applicable to detect diseases. Such nanoprobes are used for sensing or imaging, tracking the specific cells, to monitor pathogenesis, therapy and to enhance research in basic biological phenomena (Doria *et al*., 2012).

Metals like Cu, Co and Zn etc have been explored to be used in the development of biosensors (Sgadevan and Periaswamy, 2014). Magnesium ferrite nanoparticles with size 15 nm have been successfully used to sense dopamine electrochemically up to $7.7X10^{-8}$ M, other metal oxides and ferrites along with their respective electrodes have potential to sense and determine biologically active compound including neurotransmitter (Reddy *et al*., 2011). ZnO nanoparticles are inherently electro-catalytic in nature and these are utilized to immobilize enzymes. ZnO nanoparticles appear to be a good material for biosensor because this nanomaterial does not damage the biological activity (Dubey and Kumar, 2012). Hybrid NiO/ZnO nanoparticles prepared from carbon paste electrode (CPE) exhibit stable elevated electrolytic activity, surface area of electrode and sensitivity to detect lower limits up to 0.2 M Reddy *et al*., 2012). ZnO and ZnO/polyglycine have very good potential to detect dopamine, ascorbic acid and uric acid in the sera samples; it shows high electrolytic behavior which appears to be suitable for analytic chemistry and biosensor for biological system (Reddy *et al*., 2012). Flexible and self-supported microelectrodes with seamless solid/nanoporous gold/cobalt oxide hybrid structure for electrochemical non enzymatic glucose biosensors. Gold skeleton and cobalt oxide nanoparticles exhibit synergistic electro-catalytic activity with respect to glucose oxidation; multilinear detection range having ultra sensitivities at very low potential of 0.26 v/s Ag/AgCl been observed when amperometric glucose biosensors were used. Ultra low detection limit to the tune of 5M in 1s was seen having sensitivity up to 12.5mA Mm^{-1} cm^{-2} (Lang *et al*., 2013). Thus, gold micro wires provide very good electronic/ionic conductivity and mass transport for increased electrolysis. Crystalline molecular material, metal-organic-framework (MOF) have properties like ultra high porosity, tunable structure, high thermal and chemical stability, such features increase the degree of suitability of these materials to be used in storage, separation, catalysis, biochemical imaging and sensing (Lei *et al*., 2014). The MOF can be fabricated to be functionalized for signal transduction strategies like optical-electrical, electrochemical and mechanical and photo electrochemical schemes and their analytical applications can be applied for the detection of solvent molecules, metal ions, DNA, proteins etc, (Chen *et al*., 2011).

NANOWIRES

Nanowires when get associated with potential chemical and/or a recognizing part of biomolecule depending on the some favorable surface properties then the interaction of chemical binding on the respective surface bring about some change and this change is in the conductance of nanowires; the change in conductance is very sensitive, real time and quantitative in nature. This feature of nanowires has been exploited and the nanowires are being used in the development of biosensors. In the recent times, silicon nanowires field-effect transistors found to be very useful tool as a biosensor because these devices exhibit very high degree of sensitivity, selectivity, label-free and real-time features (Zeng *et al*., 2012). These field-free transistors can be made a reusable tool by subjecting them to reversible surface functionalization technique. It is very effective form *i.e*., 3D nano-field-effect transistor probe with silicon nanowires have been found to be most suitable for monitoring or recording intracellular signaling process and intracellular potentials. This engineered nano-device has been effectively used to study protein-protein interaction, DNA hybridization, monitoring viral infection, electrical and transmitter signaling among living cells and detection of early stages of cancer (Zeng *et al*., 2012). Biomarkers with reference to cancer, cardiovascular diseases and infectious diseases have been successful explored (Penner *et al*., 2012). There has been tremendous focus on the transformational betterment to enhance the performance of sensors involving nanowires; the improved forms of sensors have been used either as chemical or biosensors. Innovative fabrication of nanowires has resulted in a refined technique incorporating receptors onto and/or into nanowires (Mohamad *et al*., 2014).

Engineered nanowires are effective in detecting DNA in physiological saline (Choi *et al*., 2013). When passive oxide layer present on the nanowires was removed the sensitivity of silicon nanowires sensors was enhanced. Semiconductors nanowires and conductive polymer-nanowires could precisely detect peptides and protein cancer markers in plasma and whole blood up to the physiological concentration i.e., without any pretreatment to the samples (Choi *et al*., 2013). Biosensors and/or chemical sensors have been developed by using silicon nanowires to increase their sensitivity many folds based on the surface volume ratio and field-effect-transistor. Biosensor have been developed from a modified complex-silicon nanowires field-effect transistor and used to detect matrix metalloproteinase-2 (MMP-2) with specific focus on protein biomarkers with reference to human diseases. This involved a cleavage type of enzyme reaction involving specific peptide sequence (Schuth, 2003).

SiNWs having dimensions like width 100nm and height 100 nm were modified in to a wafer of p-type silicon-on-insulator using electron beam lithography (Mohamad, 2014). DNA-Au nanoparticles complex having negative charge was associated with specific peptide; relatively more sensitive system was created when SiNWs were used. This complex is bound with aldehyde functional SiNWs; this complex is attached with DNA whose negative charge has been increased – this enhances the change of conductance of SiNWs, this change is accomplished by involving enzymatic-cleavage of specific peptide and MMP-2. By using SiNWs-biosensor, the MMP-2 was detected up to 10 fM to 10 nM concentration (Schuth, 2003). Boron doped silicon nanowires (SiNWs) were found to facilitate the high sensitivity, real-time electricity dependent sensors and were convenient in while conducting chemical and biological investigations. Further, biotin treated SiNWs could detect streptavidin up to picomolar (pM) concentration in the given samples. The semiconductor nanowires have been a potential detector for sensitive, label-free, real-time detection of analytes in chemical and biological samples in vivo (Sagadevan and Periaswamy 2014). Biosensors fabricated using SiNWs have been exploited to detect prostate cancer risk biomarkers-8-hydroxy deoxyguanosine (8-HdG); target antibodies were grafted on the surface of SiNWs by adopting electrochemically induced functionalization technique. 8-hydroxy deoxyguanosine was detected up to 1ng/ml (3.5n M) (Mohammed, 2010).

POROUS MATERIALS AND POROUS NANOMATERIALS

Use of porous and high-surface-area in the field of templating technique is of significance and their applications in fabricating the material. The structure and texture of this material can be controlled to very high degree, when template molecules or supra molecular units are associated with the mixture of the materials to be used for the synthesis, these molecules stop the growth of solid resulting in the formation of porous system. The final form is quite close to the scaffolding (Ju *et al*., 2011). The finished set up is referred as 'endotemplate' of the porous system. In these pores other solid can be framed and this is the basis of synthesis of porous material. In case of 'exotemplate' when the process is completed the scaffolding is separated leaving behind the finely divided porous solid matter. Its formation depends on the conductivity of the template (Ju *et al*., 2011). By carefully and specifically selecting the template a porous material of required structure and texture can be fabricated within the range of nanometer to micrometer. The porous material is classified based on size of the pores formed in to three classes: micro porous (< 2 nm), meso porous (< 2-50 nm) and macro porous (> 50 nm). When porous materials exhibit pores of nanoscale the material is said to be nanoporous material. These materials exhibit large surface area, need based and controllable pore size and versatile composition. Nanoporous material is among the most sought after nanomaterials to be used in the field of bioengineering, catalysis and biosensing. The nanoporous material exhibits wide range of composition including silica, carbon, metals, metal oxide and hybrid composition (Mora *et al*., 2009).

The nanoporous materials are suitable and useful for electrochemical and optical forms of biosensors. Biosensors fabricated from nanoporous material have been successfully used to detect biomolecules like glucose, DNA, antibodies and bacteria, with higher degree of sensitivity. Parameters like thickness, refractive index, pore-size etc., of porous silicon material can be modulated in a controlled manner and be accomplished involving adjustments in the anodizing conditions (Martin-Palma *et al*., 2008). Mostly the biosensors are focused on either production or detection of discrete or continuous signals; these signals are in relation or in accordance to one single or many analytes that are generally related. When silicon

is used as transducer, it has the ability to convert signal with respect to analyte into optical or electrical signal. Further, large surface area helps to get associated with biological analytes but a problem may exist related to a possible high reactivity with environment; this in turn may result in degradation of biosensor and/or may impart false positive signal. This condition can be counter acted by stabilizing porous silicon surface with the help of surface chemistry. The binding ability of silicon biosensors can be improved by functionalization using methods like oxidation (Martin et al, 2011); silanization (Sheltzer *et al*., 2002), hydrosilylation of alkenes and alkynes (Kilian *et al.*, 2009) radiation or other chemical approaches (Chen *et al*., 2004).

MAGNETIC NANOPARTICLES

Magnetic nanoparticles are versatile and suitable diagnostic tools in the biological and medical fields (Zhang et al, 2009). Magnetic biosensors are fabricated in different forms like single domain or super paramagnetic (Fe_3O_4), gerigite (Fe_3S_4), maghemite (γ-Fe_2O_3), types of ferrites (MeO-Fe_2O_3); in these ferrite Me can be replaced by Ni, Co, Mg, Zn or Mn etc. These nanoparticles identify biorecognising molecules and get bound to them, thus, these biomolecules are separated or get enriched and can be easily detected. Some of the earlier techniques include magnetic cell separation which involves magnetic field gradients and is capable of manipulating and isolating cells which are magnetically labeled, similarly magnetically labeled targets either antibodies or other molecules can be detected using magnetic meter. Another technique is based on high-transition temperature DC superconducting quantum interference device (SQUID) and is quite appropriate to detect biological molecules associated with super paramagnetic nanoparticles. The basic concept of this technique involves Mylar film along with target molecules and treatment with magnetic nanoparticles. The specific magnetic properties of magnetic nanoparticles (MNPs) have been involved in the development of biosensors, the same have been suitably applied in the studies of hyperthermia, magnetic resonance imaging (MRI), contrast agent, repairs of the tissues i.e. cell growth and cell sheet harvesting, immunoassay, drug/gene delivery, cell separation, giant magneto resistance (GMR) sensors (Tuutijarvi et al, 2009). With the help of maghemite (γ-Fe_2O_3) nanoparticles As(v) were separated from the aquatic media up to 50mg/g level. These maghemite nanoparticles may be exploited to fight As (v) pollution (Beratella *et al*., 2013). Oxidase enzyme dependent interaction can be investigated by using surface active maghemite nanoparticles (SAMNs) having 10nm size in the form of carbon paste. These nanoparticles acts as efficient H_2O_2 electro-catalyst, when coupled with H_2O_2 produce the enzyme and may be considered as a reagent less biosensor device (Zhou *et al*., 2014). Magnetic nanoparticles as a material for the biosensors have been quite versatile and effective for the detection of medicinal and biological analytes; further, depending on the high transition temperature DC super conducting quantum interference device (SQUID) super paramagnetic nanoparticles can be exploited to detect biological molecules (Sgadevan and Periaswamy, 2014). The super conducting quantum interference device (SQUID) technique involves a basic concept in which a target is bound to Myler film (referred as polyester film/plastic sheet) and it is kept under microscope. A suspension containing magnetic nanoparticles and antibodies is added to the well on the Myler film; magnetic field of 1-s pulses is applied and this is arranged parallel to the SQUID (Zhou *et al*., 2014). When the field is aligned these nanoparticles form magnetization net. When the field is turn off the magnetization net relaxes. The unbound nanoparticles undergo 'Brownian rotation' and result in the development of no detectable/measurable signal. The nanoparticles bound to target are captured, these exhibit 'Neel Relaxation' (time dependent magnetic process) and results in the production of magnetic flux; this magnetic flux slowly declines and is detected by SQUID (Zhou *et al*., 2014). Small molecules like biotin, digoxigenin (a steroid found in flowers and leaves of *Digitalis purpurea*), 16-nucleotide DNA oligomers, and proteins like streptavidin(52.8 kDa) and antibodies have been successfully detected by optical inter ferromagnetic transducer in which porous silicon was used. Fabricated magnetic porous carbon microspheres have very high specific surface area and employed as catalyst to remove methylene blue from waste water. These particles could efficiently remove methylene blue up to 40mg/l level in just 40 seconds. Once the reaction was over the catalyst could be isolated from the medium within short duration of seconds by employing magnetic field externally. The catalyst maintained its activity up to ten trials of methylene blue removal (Jiri *et al*., 2014). Iron oxide magnetic species were used to functionalize carbon-nano-allotropes; these fabricated nanoparticles are of cost effective, easily available and show much better biocompatibility. This compatibility is dependent on their super paramagnetism and ability to give strong magnetic response under external magnetic field. Further, iron oxide in forms like magnetite, maghemite, hematite, when get associated with carbon nanostructures impart synergetic and cooperative effect and have been observed to increase photo-catalytic capability and electrochemical performance.

OPTIC FIBERS

Optical fibers are made from glass and plastic; these are flexible, transparent and have transparent core and are surrounded by cladding materials. The cladding materials have lower refractive index and causes light to remain within the core (Leung *et al*., 2014). Optic fibers biosensors are amongst one of the low cost, efficient, accurate and convenient to handle devices. These devices are based on the optical properties which make the detection of the biological specimens like cells, proteins, DNA etc. very convenient (Leung *et al*., 2014). The basic principle involved is based on the geometrically fabricated tapered optical fibers exposed to evanescent field *i.e*., electromagnetic wave propagating in a dielectric wave guide, the field decays exponentially away from core-cladding boundary; evanescent tail is an integral part of the guided wave; modification of the evanescent field modifies the guided waves (Phoenix photonics, 2003). Tapered fibers optical biosensors can be modified by various optical transduction mechanisms which include change in refractive index, adsorption, fluorescence and

Surface Plasmon Resonance. All these modifications enhance the efficacy of optical fibers and in turn the biosensors based on these materials (Leung *et al*., 2014). Suitable optical fiber biosensor can detect glucose concentration by using glucose oxidase (which converts glucose into gluconic acid within blood sample). This device is economic and feasible to calculate insulin dosage to be administered by using variable optic fibers with post process treatment (Edwin *et al*., 2010). A label free fiber-optic biosensor based on evanescent wave absorbance to find out the existence of analytes which may be in the form of microbe, virus, or biomolecule specifically proteins has been devised (Kundu *et al*., 2010). This device is cost effective, field applicable and user friendly too. The device was fabricated and U-bent was involved along with localized surface Plasmon resonance of noble nanoparticles at visible wave length. This set up was found to be quite efficient to detect biomolecules (Kundu *et al*., 2010). Miniaturized fiber optic biosensor has been fabricated based on adsorption transmission to detect Cd in milk. The basic principle is based on the concept that Cd ions inhibit the activity of urease enzymes; the attainable limit was found to be 0.1µg/l (Verma *et al*., 2010). Guided-wave sensing facilitates intrinsic advantage of optical techniques and it accomplishes detection more effectively and relatively unperturbed manner. Although fiber and integrated optics are basically meant for application in the field of telecommunication, these optic fibers are quite suitable for the development of biosensors (Shaikh and Patil, 2012). The primary reasons for the guided-wave optics to be considered for sensing may include non-electrical mode of separation, offers least intrinsic immunity *i.e*., it offers least interference towards radio-frequency and electromagnetic interactions. These devices are smaller in size, very light in weight and highly flexible, can easily reach the site which are otherwise inaccessible; these exhibit high resistibility to chemical aggression and ionizing media; provide secured data transmission and finally optical data is communicated uninterruptedly, quite suitable material for fabricating biosensors (Shaikh and Patil, 2012).

CARBON AND ITS FORMS

Carbon exists in many forms and has been used in varied forms in different domestic, industrial, medicinal fields. Carbon as nanomaterial is in the form of nanospheres, nanowires, single walled carbon nanotubes, multiwalled carbon nanotubes *etc*, (Kruss *et al*., 2013). Graphene, a pure form of very thin sheet (one atom thick), carbon exhibits exclusive electronic, optical, thermal, chemical and mechanical properties and because of these features it is the prime material sought after to be used in biosensors. Carbon in nanowires form exhibit very high degree of sensitivity and have ability to form strong coupled interface with cell membrane, because of these qualities nanowires are used as field-effect transistor that form bioelectronic interface of nanoscale with cells and tissues. Signals arising from spontaneous palpitation movements could be recorded from a well defined extracellular signals arising from chicken embryonic cardiomyocytes (Cohen-karni *et al*., 2010). A nanocomposites consisting of reduced graphene oxide and carbon nanowires was fabricated, in this case graphene oxide serves as a carbon source (no addition was done for carbon precursor). The device exhibited efficient detection of dopamine (Cao *et al*., 2012). A highly stable porous silicon composite material (porous template) is fabricated, crystalline Si was electrochemically anodized in HF-containing electrolyte; this product was subjected to infiltration of poly (furfuryl) alcohol (PFA) followed by carbonization, this resulted in the formation of porous silicon carbon composite (pSi-C) having optical smooth thin film. The sensitivity of the protein A-modified pSi-c sensor was found to be better in comparison to the label free porous composite constructed by using TiO_2 and Al_2O_3 optical biosensor (Chun *et al*., 2012). An amperometric malic acid nanosensor was fabricated and it was found to be fast and specific in nature. The carbon electrode was used as working component, platinum as counter component and silver as reference electrode; MWCNTs were coated as screen printed electrode after immobilizing malate dehydrogenase enzyme (Rahul *et al*., 2012). Graphene has potential to be used as a probe molecule because it helps to transfer and modify signals. It has the capacity to interact with biomolecule accurately and selectively. This ability is due to the fact that it can manipulate multi-functionalized surface chemistry; hence, it is preferred nanomaterial for detecting enzymatic, immune, gene and micromolecular biosensors (Li and Yang, 2013). MWCNTs based purine electrodes were fabricated and the immobilizing purine nucleosides were coated on MWCNTs, benzene and its mono-, di-, and poly substituted derivatives could be easily and accurately detected electrochemically. The ability of MWCNTs to be coated with graphene electrode to form a film was used investigation involving electrochemical impedance spectroscopy, cyclic voltammeter, containing redox couple. It was found that MWCNTs based purine electrodes were stable, exhibited higher degree of reproducibility, selectivity and regeneration (Gayathri *et al*., 2014).

QUANTUM DOTS

Bailey *et al.,* expressed that quantum dots can be easily excited by a single light source and as result they emit multicolored emission. Further, in the recent times it has been observed that these semi conductor particles combine covalently with biomolecules such as protein, antibodies, peptides, DNA, RNA, or small legends (Bailey *et al*., 2004). Quantum dots have been found to be a novel material to be used in the fabrication of chemical and biosensors, The properties of these materials exhibit very broad continuous absorption spectra wave length ranging from between UV to visible range with respect to the size of the particles; exhibit low degree of photobleaching; higher degree of photochemical stability; broad excitation spectra having 20-40 nm full width *etc*, (Fransco and Chanio-Takis, 2009). Basically the nature of quantum dots is colloidal, non-crystalline semiconductor; because of their optical and novel spectroscopic potential quantum dots appear to be preferred material to be used to detect multiple analytes in comparison to the traditional fluorophores. CdTe quantum dots were prepared in aqueous phase having size of few nm size; these gave very high luminescence quantum yield more than 60% and were found to be very suitable for biomedicine labeling (Ung *et al*., 2010). The quantum dots are pH sensitive and

could be used as pH detector. The fluorescence of CdTe quantum dots were employed to detect proton flux formed due to ATP synthesis in chromatophore (Ung *et al*., 2010). A specific quantum dots were used to observe surface charge on the biological cells quantitatively within nanoscale resolution. The development of such quantum dots involved amino modified CdSe/ZnS nanoparticles. In a special buffer system quantum dots were positively charged and were uniformly dispersed in aqueous medium; the surface of living cells were charged negatively, 2D/3D images of quantum dots labeled charged cells were obtained by using wide-field optical section microscopy (Huang *et al*., 2011). Small but robust silica-coated quantum dots were engineered and changed them to sensitive molecular beacon; these were employed to detect DNA. The fabricated quantum dots were stable, showed high yield of analyte within a wide pH range (1-14) and 2M concentration of salt. These quantum dots were smaller than 10 nm in diameter and their detection limit was found to be 0.1 nM and duration of detection 15 min (Chung-Shien *et al*., 2011). The study of behavior of InP/ZnS quantum dots revealed that position of peaks of the two emissions showed similar pattern as the temperature increased from 15–70 K and the band gap became narrower with the corresponding temperature. The ratio of integrated intensity of emission declined slowly and this ratio elevated fast as temperature reach beyond 80 K (Thi *et al*., 2011). Highly luminescent colloidal quantum dots were developed like CdS/ZnS or ZnSe/ZnS having their core/shell conformation made up of CdS, ZnS or ZnSe/ZnS. These were used to test agriculture-bio-medical analytes, tracking residual pesticides, detection of residual clenbuterol in meat/milk, detection of H5N1 avian influenza virus in breeding forms (Thi *et al*., 2012). Their overall performance exhibited that fabricated quantum dots showed good crystalline spherical shape emit strongly at the expected wave length between 500 and 700 nm having luminescence quantum yield 30-85% ((Thi *et al*., 2012).

DENDRIMERS AS A MATERIAL FOR BIOSENSORS

Dendrimers are one of the preferred nanocarrier systems involved in drug delivery. These nanoscaled globular molecules have been tried to be used as plausible material in the development of the biosensors (Mohamad, 2014). Basically dendrimers are macromolecules having uniform spherical/globular out line with well defined mono dispersed molecules having nano-dimensions. These nanomaterials exhibit some of the specific properties like hydrophilicity, ability to get designed easily or form different conformations having varied composition, high functional group density etc. These molecules have branching pattern showing dendritic cervices and intense branching. These are 3D molecules and have high degree of sensitivity; nonspecific binding is least (Thi *et al*., 2012). DNA-poly (amidoamine) PAMAM biosensors have been fabricated and used to detect DNA. It is nano-conjugating dendrimer and involves electrochemical impedance spectroscopy (EIS). When DNA was detected using this biosensor involving hybridization of single DNA (ssDNA) probe, it was conjugated on mercaptoacetic acid covalently using self assembled monolayer gold electrode. 4.5 (G-4.5) PAMAM-target DNA complex was formed in the solution. When double stranded DNA got associated with gold electrode then G-4.5 PAMAM get associated with carboxyls at its periphery; the fluctuations in the interfacial electron transfer resistance of electrode was recorded by electrochemical impedance spectroscopy, the probe used was $Fe(CN)_6^{3-/4-}$ redox. The detection limit of recording was found to be pH level (Satija *et al*., 2011). Dendrimers have been exploited to capture biomarkers with higher sensitivity and specificity. The specific abilities of dendrimers related to interaction have been employed to detect a wider range of cytokines, biomarkers and has been found to be suitable for multiplex micro-bead assay, conductometric immunosensors and field effect biosensors (Zhu *et al*., 2009). Dendrimers are highly stable macromolecules and have relatively higher accessibility towards the target analytes/ biomolecules and less variations while responding to biochemical fluctuations. By virtue of all these features it is preferable to design a sensor involving dendrimer resulting in enhanced sensitivity, performance and at reduced cost (Thi *et al*., 2012). Araque *et al*., 2013, prepared reduced Graphene nanoparticles from graphene oxide using two step covalent modification techniques. Modified graphene oxide was subjected to cross linking and partial reduction resulting in graphene nanoparticles with crumpled paper like appearance. The final modified graphene was used as coating material for glassy carbon electrode; the nano structured electrode was tested for electrochemical biosensor involving immobilization of enzyme tyrosinase cross linked glutaraldehyde. The observations indicated that this material is suitable for electrochemical investigations for catechol in 6 seconds duration exhibiting linear range 10 nM to µM, sensitivity $424mA^{-1}$ and stability at 4ºC in wet conditions (Han *et al*., 2014). Dendrimers-encapsulated metal nanoparticles are very suitable to be used in electrochemical biosensors. Their nanosized and dendrimer-core metal nanoparticles conformation make them very effective for electrochemical biosensors. These devices are very sensitive and efficient in measuring small biomolecules, toxic molecules, DNA and biomarkers for immune-sensing diseases (Araque *et al*., 2013).

CONCLUSION

Development of biosensors is the need of the current scenario of the growth in the scientific, industrial, biological and medical fields. It is focused on the global interest to meet the expectations of growing technology, preserving and improving health of humans, animals, crops and the environment. Biosensors are essential tools for the analytic techniques to maintain quality, quantity, safety of food, medicines, and products of industrial and common use. Efficiency and precision, easy to handle on field and/or laboratories, provide quick analysis of large number of samples, are some of the main targets for biosensors. Interaction between biosensors and receptors is very essential. This interaction has to be well understood because it holds a key role at the bottom line of the whole concept of working of biosensors. This review on biosensor, reveals that nanobiosensors are efficient, prompt and can be fabricated as per the need arises for the analysis and is relatively cost effective. Nanomaterials like

metals, metal oxides, carbon and its forms, nanowires, porous and composites, magnetic material, optical fibers, quantum dots, probably any nanosized material which can accomplish the detection of biomolecules or any other receptor can be used as a raw material in the field of development of biosensors. In spite of the fact that large number of biosensors are being explored but there is a dire need to delve for more biosensors in order to meet the huge demand from current industrial, domestic, therapeutic, biological and medical scenario.

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