ELECTROCHEMICAL BIOSENSORS AND TRANSDERMAL DRUG DELIVERY SYSTEM AS AN APPROACH TO DIAGNOSE AND TREAT HYPERTENSIVE HEART DISEASE- A REVIEW

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ABSTRACT

Developing countries are in immense need of portable and easy-to-use diagnostic tools because of the lack of access to sophisticated diagnostic centres in the villages and underdeveloped areas. Hypertensive Heart Disease is a serious health ailment, which results in various cardiac disorders, stroke and other severe cases that may result in the damage of the kidney, brain, liver, thereby, causing multiple organ damage. A few biomarkers related to the Hypertensive Heart Disease are easily found in the blood and urine. The detection techniques used at present are expensive, and detect the prevalence of the disease only after the occurrence of a few signs and symptoms. We thereby, present a review on biosensors that would be cost-effective for detection of the disease. Electrochemical biosensors detect the concentrations of the biomarkers that are present in the biological fluids to confirm the prevalence of the disease. Once the biomarker concentrations cross the threshold value, the disease is confirmed, which then needs to be treated. The treatment for the disease could employ Transdermal Drug Delivery System, which serves to be one of the most convenient methods of drug administration. We hence present a review of Electrochemical Biosensors that could detect Hypertensive Heart Disease and the Transdermal Drug Delivery System, which could be used for the treatment of the disease.

Keywords: Hypertensive Heart Disease; electrochemical biosensor; transdermal drug delivery system.

I. INTRODUCTION

Rapid on-site detection techniques and point-of-care diagnostics are a basic necessity in the underdeveloped countries so that an untrained and unskilled person can easily use the devices for detecting the biomarkers [1]. Early diagnosis of cardiovascular diseases has consequently become highly important to decrease the mortality rate and it is believed that hypertension and cardiovascular diseases account for 80% of global deaths and contribute to >85% deaths in India [2,3]. Hypertensive Heart Disease is a serious cardiac ailment that is mainly caused due to hypertension. It further leads to stroke and cardiac arrest and eventually may have serious effects on the other vital organs of the body such as liver, kidney, lungs and also the brain. The development of cost-effective sensors for easy detection of the disease and simple drug delivery systems will hence, be a boon to the population.

Presently, numerous methods are being used for the detection of the biomarkers such as ELISA [4], chemiluminescence and fluorescence techniques [5] and radioimmunoassay [6]. However, these are complicated and time-consuming methods and most importantly, require a skilled person to perform them [7,8]. The simplest way is to develop a cost-effective biosensor for detecting the relevant biomarkers and eventually come up with a drug delivery method for the administration of the required drugs into the diseased person. Electrochemical methods of detection provide many advantages such as sensitivity, selectivity, rapid-detection, portability, low price, and simplicity and have a common use for the detection of biological substances, metabolites, and drugs [9]. The most convenient and accessible site for drug administration is the skin [10] and hence, Transdermal Drug Delivery System (TDDS) turns out to be the most effective approach for delivering a required amount of drug onto healthy skin. [11,12].

II. HYPERTENSIVE HEART DISEASE (HHD)

Alteration in the coronary arteries and left ventricular arteries leads to an elevation in the blood pressure (BP) of a human being. These contributing factors result in an ailment called hypertensive heart disease. Chronic Hypertension serves to be the starting point of Hypertensive Heart Disease [13].

Table 1: Representing the range of blood pressure that draws concomitance with various stages of hypertension [14].

Category	**Optimal**	**Prehypertension**	**Stage I**	**Stage II**
Systolic BP, mm Hg	< 120	120-139	140-159	>160
Diastolic BP, mm Hg	< 80	80-89	90-99	>100

Hypertensive heart disease is inclusive of various conditions, namely heart failure, thickening of the heart muscle (left ventricular hypertrophy or LVH), narrowing of the arteries (coronary heart disease or CHD) and so on [15]. Complications in LVH and CHD can lead to heart failure, ischemic heart disease, arrhythmia, and sudden cardiac arrest. The propensity of individuals perceiving congestive heart failure, arrhythmias, myocardial infarction, and sudden cardiac death is not meagre. So, the first step in recognition of HHD forms an indispensable part in this whole cumbersome process of treatment.

The very inception of Heart Failure (HF) is Left Ventricular Dysfunctionalism (LVD). Eventually, there is an increase in the volume of the Left Atria (LA) and the walls of the LV begin to thicken, thereby leading to the diastolic dysfunction of the Left Ventricle (LV) [16,17]. Heart failure can be defined as a pathophysiological state in which heart dysfunction gives incompetence to pump blood in a needful amount to meet the metabolic requirements of the organism [18]. HF may arise due to functional and structural defects in the myocardium [15].

A. *Prevalence*

India as a country with almost 1/4th of world population offers a very grim picture. Hypertension accounts for 24% of Coronary Heart Disease (CHD) deaths and 57% of stroke deaths in India. However, the death associated with hypertension is not just conformed to India but most of the developed and developing Nations. [18].

The global systemized incidence of hypertension was about 25.9% in adults, aged above 20 years in 2000. And there was an increase in hypertension incidence by 5.2% from 2000 to 2010. In 2010, 31.1% of people suffered from hypertension [19]. The discrepancy is evident in terms of age and gender of individuals. The hypertension incidence spikes with the increase in age and are prevalent more among men than women until the age of 55 years. In India, according to 2000 data, 20.6% of women and 20.9% of men were suffering from hypertension. The World Health statistics of 2012 has estimated that 29.2% of males and 24.8% of females were gripped by this aliment [20]. Women have a higher risk of having CHD when the triglycerides level is greater. Smoking and family history are also the factors that have an impact on CHD in females than in males [21].

Table 2: Depicting the risks of cardiovascular disease with different age groups [13].

Age	Female	Male
45-54	33.2%	36.1%
55-64	55.5%	57.6%
65-74	65.8%	63.6%
> 75	81.2%	73.4%

B. *Cause & Risk factors*

The rudimentary reason for the occurrence of the disease in the adult population is because of the systolic blood pressure, which goes on a surge with incremental age. However, factors like lifestyle, inactivity, poor eating habits and diets including, an abundance of fats, and consumption of alcohol aggravate the menace [22]. Also, people suffering from pre-conditional diseases like type-2 diabetes are 1.5-2 times more prone to having hypertension [23]. Any change in DNA sequence due to sequence repeats, deletion/insertion or single nucleotide polymorphism leads to genetic polymorphism, which may lead to Cardiovascular Disease. So, one must take genetic polymorphism into consideration for the risk prediction of CVD [24]. Even environmental factors like migration to a different place, change in lifestyle and others have an important impact on health and in developing cardiovascular disease (CVD) [25]. According to WHO, 80% of deaths occur due to air pollution which results in heart disease and stroke. Even metals like cadmium, arsenic, and lead are a concern for the development of CVD [26]. Lack of social support, the stress in family life or at work, depression and low socio-economic status are also factors that may pose a risk to develop the disease [27].

C. *Treatment*

Antihypertensive medications are prescribed to lower elevated BP:

- Thiazide diuretics like chlorthalidone, indapamide are the first-line therapy for the treatment of hypertension and congestive heart failure as well as the accumulation of fluid and edema of the body caused by a condition such as heart failure, cirrhosis, and chronic kidney failure.
- Angiotensin receptor blockers (ABR) or angiotensin-converting enzyme (ACE) inhibitors also serve to be the first-line treatment for hypertension. They are used to treat several heart-related conditions, including hypertension, heart failure, heart attack and preventing kidney damage associated with hypertension and diabetes. Examples: Capoten, Vasotec, Tritace.
- Calcium channel blockers (CCB) like Norvasc, Plendil, Cardene slow down the calcium ion movements into the blood vessels walls and cells of heart, and thereby, help to widen the blood vessels and so, the heart can easily pump blood.
- Nitrates and hydralazine are vasodilators that help in improving tissue perfusion in heart failure. However, they are not used as first-line

therapy. Other drugs, like diuretics or ACE inhibitors, are usually combined with these vasodilators.

- It is beneficial to administer beta-blockers in patients with pectoris atrial fibrillation, angina and HF. They are, however, not used as initial treatment.

Beta-blockers and ACE inhibitors/ARB are preferred in younger patients (<55 years), CCB and diuretics are preferred in older patients (>55years). It is recommended that no two medications from the same class should be used for treatment, for example, using an ARB and ACE simultaneously [13,28].

D. Diagnosis

Table 3: Showing the different tests done to diagnose the disease, the methods used [16] and their average prices.

Tests	Method	Price
Electro-cardiogram	Monitors and records the heart's electrical activity.	$50 (Rs.3,000-4,000)
Echo-cardiogram	Using ultrasound, detailed picture of the heart is taken.	$60 (Rs.4,000-4,500)
Coronary angiography	The flow of blood through coronary arteries is examined.	$100 (Rs.7,000-8,000)
Nuclear stress	To examine the flow of blood into the heart.	$170 (Rs.12,000-13,000)

By cardiac imaging, the function and structure of the heart are visualized. Tests like echocardiography are useful to contribute instant information of chamber size, valve abnormalities, wall thickness, the function of LV and RV [29,30].

E. Prognosis

Cerebrovascular diseases like CHD, congestive HF, and chronic kidney disease are caused due to hypertension. Mortality rate increase when it is left untreated. Moderate hypertension is allied with atherosclerosis in 30% of people and organ damage in 50% of the people if it is left untreated. HHD depends on a varied range of components such as the prevalence of concurrent CVD and other manifestations [13,28].

F. Complications

Left undetected, high blood pressure can lead to a lot of complications. The occurrence of irregular hypertrophy may lead to a rise in oxygen demand by the myocardium, which is called Angina. High BP can also damage blood vessels in the retina, resulting in loss of vision and this condition is called Retinopathy. An irregular heartbeat can even lead to sudden death. In certain cases, blocked or narrow arteries limit the flow of blood to the brain and cause Dementia. An increase in blood pressure can also cause blood vessels to weaken and bulge, and this is called Aneurysm. The walls of arteries sometimes become hardened and thickened, which leads to stroke and heart attack. This condition is known as Atherosclerosis. In many cases, Brain stroke might occur, which causes reduction of the blood supply to the brain and that leads to loss of functionality of the brain. Also, high blood pressure might lead to kidney failure, in which blood vessels in the kidney are damaged, leading to the accumulation of toxic fluids and wastes. The calcium concentration in the urine is may also increase, leading to bone loss. The loss of bone density may thereby occur due to excessive elimination of calcium (osteoporosis) [13].

G. Biomarker and its importance

A biomarker is a biological parameter that draws an affinity towards various elements of disease, both quantitatively and qualitatively. Generally, an ideal biomarker must possess a myriad of features like [30-32]

- It should be readily available in a given sample so that the detection of the disease can be done immediately without any delay.
- It should have a degree of explicitness. It must be highly specific and must be of great importance pertaining to the disease.
- A quantifiable amount of the biomarker must be present in the sample so that it is easily detected.

Applicability of biomarkers is not limited but extended over to a much wider area. In heart failure (HF), biomarkers ideally verify the presence or absence of HF syndromes and approximate the rigor of HF and the risk of disease advancement [33].

Proteins such as ALT, AST, CK, LDH, and troponin have been an indicator of the diagnosis of acute myocardial infarction [33]. Depending on the severity of the condition it can determine, the biomarkers are classified as:

- Troponin – Most determined cardiac marker. During a heart injury, it is seen to elevate in a few hours and the elevation remains up to two weeks.
- Creatine Kinase – It helps to determine the second heart attack which occurs concisely after the first.
- Myoglobin – Along with troponin, it is used to detect a heart attack.

Other tests use biomarkers like BNP and hs-CRP [34].

H. Troponin as an important biomarker

For patients affected by acute coronary syndrome (ACS), cardiac troponins have become the first preference over other cardiac markers. Cardiac injury can be indicated by a biomarker called troponin which discharges into the bloodstream during a heart attack. Natriuretic peptide and cardiac troponin have engendered a stature in the sea of biomarkers in dealing with cardiac diseases such as heart failure, acute myocardial infarction and acute coronary syndrome [35]. For the diagnosis of ACS, measurement of cardiac troponin is the prime requisite [36]. Troponin has demonstrated its capability for the diagnosis and eventually the risk of patients with acute chest pain or angina [37].

Troponin is a protein specifically found in the heart muscle cells of the body. The troponin complex is made up of three subunits: (a) Troponin I- impede actin-myosin interaction; (b) Troponin C- binds calcium; (c) Troponin T- attaches the troponin complex to tropomyosin for facilitating contraction [38]. Tropomyosin along with troponin I, T and C is present on the actin filament [39]. In a normal person, the troponin level is non-detectable. So, elevation in the level of troponin indicates myocardium [40]. Troponin levels peaks at 12-48 hours and remains elevated for 4-10 days [41]. The regulatory proteins, cardiac troponin I and T are present in cardiac tissue. The low levels of troponin T can be detected by high sensitivity cardiac troponin test (hs-cTnT) for diagnosing heart attack more quickly. The normal range for hs-cTnT is 14 ng/l. Heart attack or heart damage is most likely when the hs-cTnT test detects the level above 14 ng/l. The normal range for cTnI is below 0.04 ng/ml. When the level increases above 0.04 ng/ml, a person is more likely to have heart attack [42-44].

Table 4: Representing the range of cTnT and cTnI, that can indicate problems with the heart [34].

Troponin T	**Troponin I**
99th percentile limits-0.01 ng/ml	99th percentile limits-0.04 ng/ml
Assay range – 0.01-25ng/ml	Assay range – 0.04-40ng/ml

After MI, elevation in Troponin T and I can persist for up to 10 days. Hence, it has a positive utility for diagnosing MI. Troponin discharge can also be triggered by other conditions that cause myocardial damage [39,45,46]. Troponin C doesn't have any cardiac specificity. Therefore, it is not used in the diagnosis of MI. Compare to CK-MB, Troponin I and T assays are more specific and sensitive. [47,48].

I. Creatine kinase-MB as a cardiac biomarker

Creatine Kinase is an enzyme composed of M or/and B subunit. Three different isoenzymes are CK-BB, CK-MB, CK-BB. CK-MM is an isoenzyme specific to the muscles, CK-BB is an isoenzyme specific to the brain and CK-MB is an isoenzyme specific to the heart. The testimonial range for CK is approximately 60-140 IU/L for women and 80-200IU/L for men. There are various other reasons for the elevation of the CK-MB range. So, CK-MB is not a very specific biomarker for myocardial infarction [49], however, it is used in the confirmatory tests, to confirm the prevalence of the disease.

The level of serum and uric acid elucidate to be an independent indicator of evolving hypertension [50].

Table 5: Showing the summary of cardiac biomarkers

Test	Onset	Peak	Duration
CK/CK-MB	4-8 hours	18-24 hours	36-48 hours
Troponin	3-12 hours	18-24 hours	Up to 10 days
Myoglobin	1-4 hours	6-7 hours	24 hours
LDH	6-12 hours	24-48 hours	6-8 days

The diagnosis of HHD is usually done after the patient suffers from one of the symptoms of the disease. However, in many cases, lack of early diagnosis and treatment causes extreme complications and eventually, leads to death. Hence, there is a necessity for the early diagnosis and treatment of the disease. Biosensors now have major applications in the diagnosis of the disease. Therefore, we present a review on cost-effective biosensors that would be useful for the detection of the biomarkers for HHD.

III. ELECTROCHEMICAL BIOSENSOR

The biosensors responsible for transforming any form of biochemical information into a signal that can be analysed are called electrochemical biosensors. The biochemical information is generally in forms of analyte concentrations, like antibody or enzyme concentration and the analytical signal is in the form of current, voltage or resistance signal. They allow detecting the lower levels of specific analytes in body fluids namely blood, urine or saliva and provide a sensitive approach for direct measurement and analysis. In a typical electrochemical biosensor system, three key components are integrated to design the biosensor. These components include (i) a recognition element that interacts with the analyte (ii) a transducer that generates a signal due to the interaction, this signal can be directly measured (iii) an electronic system that is used to display, analyse and manage the data derived [51].

Many techniques can be used for electrochemical biosensing:

i) Impedimetric: the surface of the electrodes is immobilized by different biolayers and the impedance of the system is measured.
ii) Potentiometric: the biolayers immobilized in the electrode surface help in the measurement of the potential variations in the circuit.
iii) Voltammetric: the current-potential relationship in the system is directly measured.
iv) Amperometric: the redox reactions at the immobilized electrode surfaces help to measure the currents.

Relative simplicity is the main advantage of the electrochemical biosensors and they, therefore, find applications in numerous fields like disease diagnosis, environmental monitoring of hazards, telemedicine, food quality, and safety and drug formulation and discovery [52]. Clark and Lyons invented the glucose meter in 1962, which began the era of biosensors and from then onwards, the advancement in this field accelerated at an uncontrollable pace [53].

A. Mechanism of action of Electrochemical Biosensors for Detection of Disease

The biomarkers of the disease are taken as analytes and their respective receptor molecules are immobilised on the surface of the electrodes. Generally, different

components are used for attaching the receptor molecules to the electrode surface. These components help in surface modification and cross-linking [1]. Examples include polymeric molecules like DSP, nanohybrids and other linkers like APTES. Nanohybrids are known to have very good conducting properties and hence, they can be used as mediators. The enhancement of the electron flow by these nano-hybrids increases the efficiency of the biosensors [54-57].

When a sample containing the analyte is fed into the biosensor, the receptor molecules interact with the analytes, which causes a biochemical transformation. This results in the generation of an analytical signal and the transducer sends this signal to an electronic system. Amplifiers are also used in certain cases to amplify a given signal This electronic system records the data and further helps in the analysis and management of the data.

B. Biosensors employed for HHD Markers

Relevant biomarkers such as Troponin T and I and Creatine Kinase MB can be detected by using electrochemical biosensors. The respective receptor molecules can be immobilized onto the surface of the electrode, and the interactions of the biocomponents can help for analyzing the concentration present in the given sample, thereby helping in early detection of the disease. The biosensors are usually fabricated using various types of nanomaterials namely, nanowires, various metal nanoparticles, and nanotubes. However, various polymer-based fabrications of sensors are presently being done. Modifications are done using polymers to increase the efficiency of the biosensors to a great extent mainly by improving the specificity and selectivity properties, increasing the sensitivity and also providing a greater surface area [58-62].

The performance of the biosensors is solely dependent on the materials used for the making and fabrication of the electrodes. Various factors like mechanical properties, electrical conductivity, potential range, toxicity, surface reproducibility, and cost must be taken into consideration while selecting the material. Solid, noble metal electrodes are the most preferred ones, like gold, platinum, and carbon. Several studies have used Gold screen-printed electrodes (Au-SPE) for detecting the biomarkers of different cardiovascular diseases [63,64].

Metal nanoparticles-based fabrications of sensors are presently being extensively researched in the various domains of science, mainly because of their excellent features [65].

Table 6: Representing the electrode potential of different elements at 25°C (77°F). Silver, carbon, platinum and gold have positive electrode potential and hence, they serve to be highly stable and conductive materials for constructing the biosensors [74].

Element	Standard electrode potential [V]
Anodic end (+), active (this is where the corrosion occurs)	
Lithium	-3.045
Potassium	-2.920
Sodium	-2.712
Magnesium	-2.340
Beryllium	-1.700
Aluminium	-1.670
Manganese	-1.050
Zinc	-0.762
Chromium	-0.744
Iron; mild steel	-0.440
Cadmium	-0.402
Yellow Brass	-0.350
50-50 Tin-Lead solder	-0.325
Cobalt	-0.277
Copper	+0.340
Mercury	+0.789
Silver	+0.799
Carbon	+0.810
Platinum	+1.200
Gold	+1.420
Cathodic end (-), passive (no corrosion here)	

i) Noble nanomaterials: Platinum, silver, and gold are commonly used for drug delivery, therapeutics, biomolecule recognition, molecular diagnostics and imaging. They have good optoelectronics features which make them highly useful in the biomedical field.

ii) Carbon nanomaterials: they are chemically stable, have a high surface area-to-volume ratio, greater electrical conductivity, highly sensitive, very good biocompatibility and have robust mechanical strength [66,67]. Carbon nanotubes can easily detect a very low concentration of analytes [68,69]. Graphene has unique properties of 2D-material [70] and proves to be a novel material for biosensor fabrication.
iii) Gold nanomaterials: They are noble metals having very high stability and surface-to-volume ratio, very good biocompatibility and can be easily synthesized. They are extensively used in the biomedical field because of their low toxicity properties and most importantly, they do not interact with the biocomponents (e.g., biomarkers) [71].
iv) Silver nanomaterials: These are very important materials in biosensing technology. They have potential applications in diagnostic platforms for detecting biomarkers, infectious organisms and other physiological threats [72,73].

C. Detection Techniques

Electrochemical transducers are connected to biosensors to provide analytical information. The main principle is that the oxidation-reduction reactions of the analyte cause changes in the properties of the solution by the consumption or production of electron species. This change is measured by the working electrode after referring to the reference electrode, where no change is seen and the solution properties are stable. However, not all sensors make use of redox reactions and electron flow. Such sensors measure the parameters like impedance, capacitance or resistance. Biosensors mainly focus on the analysis due to changes on the surface of the electrodes deriving from molecular interactions and biofunctionalization of the surface. A few examples of such interactions include enzyme-substrate, antigen-antibody, ligand-receptor reactions and others. [62]

i) Impedimetric detection: Electrodes are surface modified by biological components and the structure and function of the electrodes are analyzed by a method called EIS or Electrochemical Impedance Spectroscopy [75]. Label-free interactions and biorecognition of the immobilized biomolecules on the sensor surface are detected by EIS [76]. Impedance Construction of EIS sensors is by using a conducting polymer as a base layer or by SAM (self-assembled monolayer) [77]. However, the main disadvantage is that false-positive results can be obtained, which can be rectified by making the non-specific binding sites of the sensor unavailable, using components like BSA protein [78].
ii) Potentiometric detection: The two electrodes in the system have a potential difference or electromotive force (EMF), which is measured at a zero-current value [78]. This potential difference arises due to the interactions of the biomolecules and the Nernst equation can be used for describing the reaction. Detection of minute changes is possible as the response of the concentration is logarithmic [76,79].
iii) Voltammetric detection: This is extensively used by researchers for biosensing analysis and it measures the relationship between the potential and the current in the system. The measurement of the potential takes place in a condition where no current is applied [77]. Voltammetric detection can be done using AC voltammetry, Cyclic voltammetry (CV), Linear Sweep voltammetry (LSV) and Differential Pulse voltammetry (DPV) methods. The only difference between these methods lies in the way the potential is applied [79].
iv) Amperometric detection: Redox reaction of the biolayers produces a current which is directly measured. The product here must, therefore, undergo the redox process and hence, be electroactive [77]. The glucose meter is the best example of a successful amperometric biosensor, which has a rapid analysis feature. This portable and effective biosensor helps diabetic patients to easily check their glucose levels in the blood [53,80].

With the advent of these methodologies, researchers aim to apply these rapid detection techniques to combat this

life-threatening disease. However, the aim is not only to diagnose the disease at an early stage but also to deliver and administer immediate therapeutics, so that the disease can be treated effectively. Transdermal Drug Delivery System proves to be one of the finest and easiest methods for drug administration and researchers are now trying to use the Transdermal Patch, to treat numerous diseases.

IV. TRANSDERMAL DRUG DELIVERY SYSTEM

Transdermal Drug Delivery System (TDDS) is a novel drug delivery system that has the potential to improve the safety and efficacy of old drugs and to rectify the problems of conventional drug delivery systems such as oral, intravenous and intramuscular [81]. Oral drug delivery (ODD) is one of the most commonly preferred methods for drug administration, but the main disadvantages of this method include drug degeneration, poor bioaccumulation of the drug, first-pass metabolism, etc in the gastrointestinal tract. For drug administration, the transdermal system is one of the approaching methods used in the current scenario [82]. Also known as "medicated adhesive patches", it aims to deliver a relevant concentration or amount of drug through the skin at a very controlled rate into the systemic circulation [83].

The major benefits include improving bioavailability, painless and non-invasive drug delivery, providing a controlled release of the medication, reduced side effects, reducing first-pass effect and drug degradation, reduction in the frequency of the dosage, reduced fluctuation in circulating drug levels and more uniform effect of the drug [84,85]. The traditional drug delivery system implicates the absorption of the drug across a biological membrane. In the Transdermal Drug Delivery System, on the other hand, the drug is released in a dosage form. This system helps in preventing any damage to the healthy tissue by maintaining the required drug levels in plasma and tissue into the body.[86].

The patch is incorporated with a porous membrane which in turn serves as a mask for a reservoir of drug solution which is further enclosed by the adhesive. When the patches are directly applied to the skin, the drug in the reservoir is released into the systemic circulation at a controlled rate via a diffusion process. Use of microneedles, suitable formulations, nanoparticles, carriers, penetration enhancers, and others further enhance the drug administration and these systems are now being used for treating neurological disorders namely, Parkinson's disease and certain skin diseases [87-89].

Skin act as a protective barrier for the and transdermal delivery requires to overcome the skin barrier to deliver the drug. This can be attained by utilizing the following two technologies: Passive and Active. Passive technologies make use of systems such as microemulsions, electrospun nanofiber, liposomes as carriers for steroids and vaccines or nanoparticles. On the other hand, active technologies use electroporation, microneedles, iontophoresis or magnetospheres [90,91].

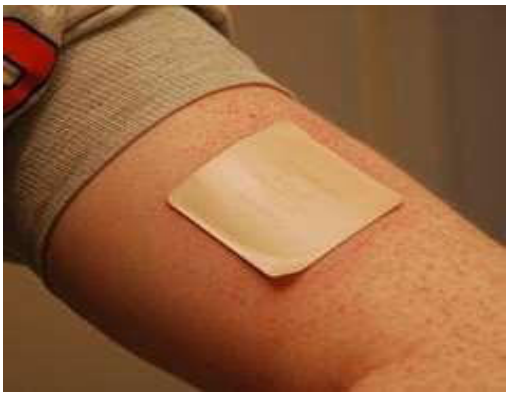

Fig-1: Representing application of transdermal patch onto the skin [84]

A. Skin

Skin is divided into three zones: (a) Hypodermis, the innermost layer, (b) Dermis, the middle layer, and (c) Epidermis, the outermost layer [92].

i) Hypodermis: Also called the subcutaneous layer, it is the innermost layer beneath the dermis, consisting of fat cells along with the sensory neurons, blood vessels, and hair follicles. It helps in providing mechanical protection and nutritional support and also regulating temperature.

ii) Dermis: The middle layer, composed of two connective tissue layers- (i) Papillary layer- upper sublayer which consists of large number of nerve fibres, capillaries, water, cells and loosely connected tissue (ii)

Reticular layer- lower sublayer, dense and thicker networks of connective tissue

iii) Epidermis: This is the skin's outermost layer and is composed of keratinocytes (which account for 95% of cells), Merkel cells, Langerhans, and melanocytes [91]. The epidermis is sub-divided into 5 layers i.e. stratum granulosum, stratum spinosum, stratum lucidum, stratum corneum and stratum basal [93-99].

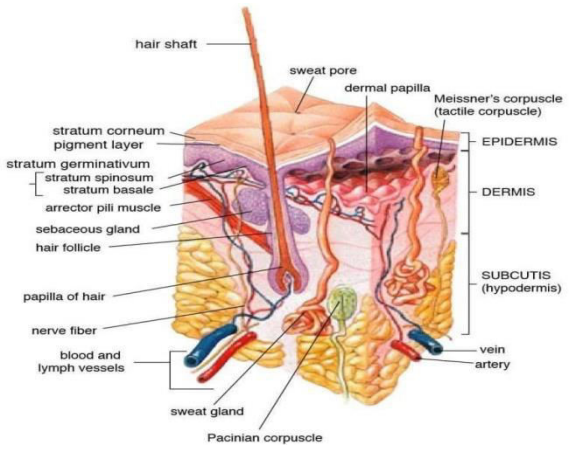

Fig-2: Illustration of various layer of skin structure of human namely: Epidermis, Dermis and Hypodermis [96].

B. Route of drug penetration through the skin

To reach systemic circulation, the drug must penetrate through all three layers of skin. There are two main routes, the Transappendageal route and the Transepidermal route [100].

In the Transepidermal route, the drug passes across the stratum corneum layer of the skin via: (a) Transcellular pathway: that involves drug transport through corneocytes, which are differentiated keratinocytes, as well as the phospholipid membranes. Firstly, the drug has to pass through each cell of lipophilic layers and then through the dead keratinocytes. (b) Intercellular pathway: that involves the delivery of drugs through the small intercellular spaces of the skin thus, making the route more complex [101-103].

The Transappendageal route mainly involves diffusion of drugs via the sweat glands and hair follicle and researchers have reported that it is an efficient pathway for transportation of large and water-soluble drugs [104,105].

The Transfollicular route has also gained a lot of attention and it is being focussed upon for drug administration via nanocarriers, which can penetrate through the hair follicle openings and reach the depth of the tissues of the skin [105-107].

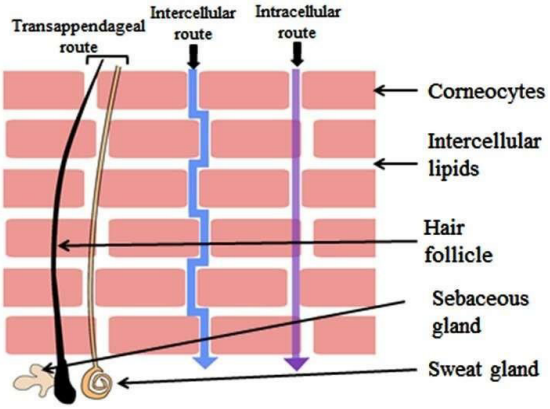

Fig-3: Representing routes of drug penetration across SC layer of skin namely transappendageal, intercellular and intracellular routes [107]

C. Transdermal permeation

Passive diffusion is the main principle for the permeation of drugs. The mechanism involves drug diffusion through the transepidermal layer and absorption by the transappendageal layer (sebaceous glands and follicular epithelium) and finally, it enters the stratum corneum.

Steps involved in the passing of the drug [81,108,109]:

• Passing of drug to rate-controlling membrane from the patch.

• The disintegration of the drug within the formulation and release from it.

• Segregation occurs in the skin's outmost layer, the stratum corneum.

• Dispersion of the drug via a lipidic intercellular pathway in the stratum corneum.

• Penetration of drug through the viable epidermis layer and in the upper dermis layer- papillary layer.

D. Components of Transdermal Patch [110-113]

i) Polymer matrix: Controls the release of the drug from the system thus, serving as a backbone for TDDS. The drug is dispersed in solid or liquid state polymer and the matrix is made. The polymer for patch should be biocompatible, non-reactive with the drug and other components of the system, provide effective release of drug and its degradation products must be non-antigenic and non-toxic to the host. Natural polymers (Gelatin, starch, cellulose, etc.) or Synthetic polymers (polyacrylate, polyethylene, polyvinyl chloride, etc.) can be used.

ii) Drug: It should be potent with lower molecular weight (>1000 dalton), and have an affinity for both hydrophilic and lipophilic layers.

iii) Permeation enhancer: By altering the proteins and lipid layer, they promote permeability. Solvents like alcohol and pyrrolidone, Chemicals like urea and Surfactants (both anionic and cationic) like Sodium Lauryl Sulphate are used.

iv) Pressure Sensitive Adhesives (PSA): It helps to maintain contact between the surface of the skin and transdermal patch. Polysiloxanes, polyacrylate, polyisobutylene-based adhesives, etc. are extensively used. The PSAs should aggressively adhere to the skin, compatible with drugs, enhancers, and excipients of the device. Drug penetration should not be affected while removing the PSAs. During the removal procedure, it should not leave any unwashable residue and sensitize the skin.

v) Backing membrane: This membrane protects the product from the outer environment as they are flexible and impermeable. Examples for backing membranes: adhesive foam pad with occlusive base plate, metallic plastic laminate, plastic baking with an absorbent pad, etc.

vi) Release Liner: The liner protects the product during storage, which is removed before use. It is generally composed of Teflon or silicone coating and a base layer.

vii) Other excipients depending upon the drug and the disease to be treated.

The transdermal drug delivery system has become a recent trend for incorporating the drug into the body via the skin, without rupturing the skin membrane and transdermal route. The drugs showing unstable GI conditions and hepatic first-pass metabolism are suitable candidates for TDDS.

E. Current Applications of Transdermal Patch

i) Nicotine patches: Nicotine patches are used to deliver nicotine through the skin to reduce the tendency of an individual to consume tobacco. Tobacco contains nicotine, which is addictive. When these patches are applied to the skin, nicotine is released and absorbed through the skin. The released nicotine binds to the receptors of nicotine in the body due to which nicotine cravings and withdrawal symptoms like anger, irritability, anxiety, depression, etc, are reduced, leading to smoking cessation. Patches are available in various dosage range, allowing the user to use it following their addiction, strongest patches to be used by the higher dependent smokers and lower to be used by the lower dependent smokers [114].

ii) Ortho Evra (Estrogen patches): Menopause, as well as post-menopausal, are treated by using estrogen patches. The patch has three layers: the inner release liner- must be removed before applying, a layer consisting of hormones, and an outer protective layer. when the patch is applied on the skin, hormones are released and absorbed to provide a continuous flow of hormones during menstrual cycles [115].

iii) Nitroglycerine patches: People with coronary artery disease (narrowing of the blood vessels) may forbid episodes of angina (chest pain) using these patches. Nitroglycerine belongs to a class of drugs known as nitrates. Angina occurs when the heart muscle does not receive enough blood. When the heart muscle does not receive enough blood, angina occurs. When the patch is applied on the skin, nitroglycerine is released, which is absorbed by the body. The drug allows the blood to flow easily to the heart by relaxing and widening the blood vessels [116].

iv) Microneedles: Microneedles are micro-scale needles used to deliver vaccines or other drugs through the skin

using the transdermal application. This can be formulated through various methods that involve photolithography or micro-molding. When the transdermal patch containing microneedles is applied to the skin of the patients, drugs are released on the target site. Microneedles can be categorized into four: solid microneedles, drug-coated microneedles, dissolving microneedles and hollow microneedles.

- Solid microneedles are used to create pores in the skin surface, the drug is delivered by applying on the skin which diffuses through the pores into the body.
- Drug-coated microneedles refer to microneedles coated by a drug using water-soluble formulation. When the patch containing this type of microneedles is applied on the skin, the drug coating is dissolved into the body, after which microneedles are released.
- Dissolving microneedles encloses the drug. The microneedles are made up of water-soluble or biodegradable polymer. When the patch containing these microneedles is applied into the skin, the microneedles dissolve completely which in turn releases the drug into the body.
- Hollow microneedles are used for the delivery of liquid drugs into the body through the skin [117].

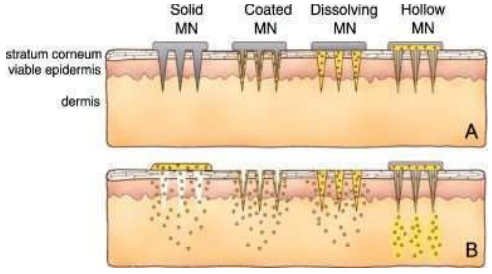

Fig-4: Representation of different types of microneedles(MN) such as solid MN, coated MN, dissolving MN and hollow MN and their mechanism [117]

Effective drug delivery method is very important for the treatment of the disease and TDDS serves to be an effective method because it is convenient, user-friendly and painless [118]. Rapid and easier drug administration procedures not only help in effectively treating the disease but also plays an important role in preventing complications that are associated with the disease.

V. CONCLUSION

Hypertensive Heart Disease involves a lot of risks and complications, which if untreated, may cost a person's life. Such a life-threatening disease requires early, accurate and rapid diagnosis and treatment. The prevalence of cardiac damage is confirmed using Troponin T and I and Creatine kinase-MB biomarkers. The detection of these cardiac-specific biomarkers with the help of biosensors electrochemically helps in the rapid diagnosis of the disease. The biosensors are user-friendly and portable and such point-of-care devices are a boon to the rural population, who lack access to good medical facilities and sophisticated laboratories. The aim, however, does not only focus on the rapid detection of the disease but also an effective treatment for the disease. Transdermal Drug Delivery System proves to be one of the best methods to administer the relevant concentration of the drug in a controlled fashion. The user-friendly, portable and painless drug delivery patch can give immediate relief to the diseased person in one stroke and therefore, help in increasing the survival rate of the diseased person, especially in cases of complications such as heart attack and stroke. Soon, POC and lab-on-a-chip devices, as well as Transdermal Patches, will be used for the treatment of different cardiovascular diseases, including HHD. However, adequate funding and investment are necessary for supporting the mechanism that is needed for the commercialization of these devices.

REFERENCES

[1] Sivashankar, S., Salama, K. N., Buttner, U., & Sapsanis, C. (2015). Flexible low-cost cardiovascular risk marker biosensor for point-of-care applications. Electronics Letters, 51(22), 1746–1748. doi:10.1049/el.2015.2371

[2] Gaziano T, Reddy KS, Paccaud F, et al. Cardiovascular Disease. In: Jamison DT, Breman JG, Measham AR, et al., editors. Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2006. Chapter 33. Available from: https://www.ncbi.nlm.nih.gov/books/NBK11767/ Co-published by Oxford University Press, New York.

[3] Abdul-Aziz, A. A., Desikan, P., Prabhakaran, D., & Schroeder, L. F. (2019). Tackling the Burden of Cardiovascular Diseases in India. Circulation: Cardiovascular Quality and Outcomes, 12(4). doi:10.1161/circoutcomes.118.005195

[4] J.Wang, A. Ibáñez, M. P Chatrathi, A. Escarpa, Electrochemical Enzyme Immunoassays on Microchip Platforms, Anal. Chem., 73 (2001), 5323–5327. doi:10.1021/AC010808H.

[5] M.I. Mohammed, M.P.Y. Desmulliez, Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers: a review, Lab Chip. 11 (2011) 569–595. doi:10.1039/C0LC00204F.

[6] F.S. Apple, A. Falahati, P.R. Paulsen, E.A. Miller, S.W. Sharkey, Improved detection of minor ischemic myocardial injury with measurement of serum cardiac troponin I., Clin. Chem. 43 (1997) 2047–51

[7] M.A. Grachev, L.E. Matveev, E.K. Pressman, V. V Roschke, A rapid method for myoglobin radioimmunoanalysis as a diagnostic tool in myocardial infarction., Clin. Chim. Acta. 124 (1982) 235–8.

[8] T. Olsson, K. Bergström, A. Thore, Chemiluminescent immunosorbent assay of serum myoglobin based on the luminol reaction., Clin. Chim. Acta. 138 (1984) 31–40.

[9] Bakirhan, N. K., Ozcelikay, G., & Ozkan, S. A. (2018). Recent progress on the sensitive detection of cardiovascular disease markers by electrochemical-based biosensors. Journal of Pharmaceutical and Biomedical Analysis, 159, 406–424. doi:10.1016/j.jpba.2018.07.021

[10] Alkilani, A., McCrudden, M. T., & Donnelly, R. (2015). Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the Stratum Corneum. Pharmaceutics, 7(4), 438–470. doi:10.3390/pharmaceutics7040438

[11] Han T., Das D.B. Potential of Combined Ultrasound and Microneedles for Enhanced Transdermal Drug Permeation: A Review. Eur. J. Pharm. Biopharm. 2015;89:312–328. doi: 10.1016/j.ejpb.2014.12.020.

[12] Schoellhammer C.M., Blankschtein D., Langer R. Skin Permeabilization for Transdermal Drug Delivery: Recent Advances and Future Prospects. Expert Opin. Drug Deliv. 2014;11:393–407. doi: 10.1517/17425247.2014.875528.

[13] Tackling G, Borhade MB. Hypertensive Heart Disease. (2019). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539800/.

[14] Kamran Riaz (Author) and Aqeel Ahmed (Co-authors). Hypertensive Heart disease (2014).

[15] Inamdar, A., & Inamdar, A. (2016). *Heart Failure: Diagnosis, Management and Utilization. Journal of Clinical Medicine, 5(7), 62.* doi:10.3390/jcm5070062.

[16] Diamond, J., Phillips, R. Hypertensive Heart Disease. Hypertens Res 28, 191–202 (2005) doi:10.1291/hypres.28.191.

[17] Anchala, R., Kannuri, N. K., Pant, H., Khan, H., Franco, O. H., Di Angelantonio, E., & Prabhakaran, D. (2014). Hypertension in India. Journal of Hypertension, 32(6), 1170–1177. doi:10.1097/hjh.0000000000000146.

[18] Kishore, J., Gupta, N., Kohli, C., & Kumar, N. (2016). Prevalence of Hypertension and Determination of Its Risk Factors in Rural Delhi. International Journal of Hypertension, 2016, 1–6. doi:10.1155/2016/7962595.

[19] Katherine T. Mills, Joshua D. Bundy, Tanika N. Kelly, Jennifer E. Reed, Patricia M. Kearney, Kristi Reynolds, Jing Chen and Jiang He. Global Disparities of Hypertension Prevalence and control. https://doi.org/10.1161/CIRCULATIONAHA.115.018912Circulation. 2016; 134:441–450.

[20] Kumar, J. (2013). Epidemiology of hypertension. Clinical Queries: Nephrology, 2(2), 56–61. doi: 10.1016/j.cqn.2013.04.005.

[21] Roeters van Lennep, J. (2002). Risk factors for coronary heart disease: implications of gender. Cardiovascular Research, 53(3), 538–549. doi:10.1016/s0008-6363(01)00388-1.

[22] Prasad DS, Kabir Z, Dash AK, Das BC. Cardiovascular risk factors in developing countries: A review of clinico-epidemiological evidence. CVD Prev Control 2010; 5:115-23.

[23] Fotoula Babatsikou, Assimina Zavitsanou (2010). Health Science Journal, Epidemiology of hypertension in the elderly 30. pp:24-30 E-ISSN:1791-809X.

[24] Sushil Gupta1, Ramesh Gudapati2, Kumar Gaurav3, Manoj Bhise (2013). Emerging risk factors for cardiovascular diseases: Indian context. DOI: 10.4103/2230-8210.117212.

[25] Aruni Bhatnagar (2017). Environmental Determinants of Cardiovascular Disease. https://doi.org/10.1161/CIRCRESAHA.117.306458Circulation Research. 2017; 121:162–180.

[26] Cosselman, K. E., Navas-Acien, A., & Kaufman, J. D. (2015). Environmental factors in cardiovascular disease. Nature Reviews Cardiology, 12(11), 627–642. doi:10.1038/nrcardio.2015.152.

[27] Christian Albus (2010). Psychological and social factors in coronary heart disease. https://doi.org/10.3109/07853890.2010.515605.

[28] MAYNARD, S. J. (2000). Troponin T or troponin I as cardiac markers in ischaemic heart disease. Heart, 83(4), 371–373. doi:10.1136/heart.83.4.371.

[29] Alexander E. Berezin. Journal of Laboratory and Precision Medicine (2018). Circulating biomarkers in heart failure: diagnostic and prognostic importance.

[30] Taguchi, J., & Freis, E. D. (1974). Partial Reduction of Blood Pressure and Prevention of Complications in Hypertension. New England Journal of Medicine, 291(7), 329–331. doi:10.1056/nejm197408152910703.

[31] Beevers, D. G., & Robertson, J. I. S. (2007). A Short History of the Study of Hypertension. Comprehensive Hypertension, 3–20. doi:10.1016/b978-0-323-03961-1.500040.

[32] Sunil K Nadar and Muhammed Mujtaba Shaikh. Department of Medicine, Sultan Qaboos University Hospital, Muscat, Oman. Biomarkers in Routine Heart Failure Clinical Care. Citation: Cardiac

Failure Review 2019;5(1): 506.DOI: https://doi.org/10.15420/cfr.2018.27.2.

[33] Van Kimmenade, R. R. J., & Januzzi, J. L. (2011). Emerging Biomarkers in Heart Failure. Clinical Chemistry, 58(1), 127–138. doi:10.1373/clinchem.2011.165720.

[34] Aydin, S., Ugur, K., Aydin, S., Sahin, İ., & Yardim, M. (2019). Biomarkers in acute myocardial infarction: current perspectives. Vascular Health and Risk Management, Volume 15, 1–10. doi:10.2147/vhrm.s166157.

[35] Hafidh A Al-Hadi, Keith A Fox (2009). Cardiac Markers in the Early Diagnosis and Management of Patients with Acute Coronary Syndrome.

[36] Mair, J., Jaffe, A., Apple, F., & Lindahl, B. (2015). Cardiac Biomarkers. Disease Markers, 2015, 1–3. doi:10.1155/2015/370569.

[37] Kitamura, M., Hata, N., Takayama, T., Hirayama, A., Ogawa, M., Yamashina, A., … Seino, Y. (2015). Different characteristics of cardiac biomarkers to decide and predict the culprit lesions in patients with suspicious acute coronary syndrome. Heart and Vessels, 31(6), 907–917. doi:10.1007/s00380-015-0698-5.

[38] MAYNARD, S. J. (2000). Troponin T or troponin I as cardiac markers in ischaemic heart disease. Heart, 83(4), 371–373. doi:10.1136/heart.83.4.371.

[39] Melissa A Daubert and Allen Jeremias. Vasc Health Risk Manag. 2010; 6: 691–699.doi: 10.2147/vhrm. s5306. The utility of troponin measurement to detect myocardial infarction: review of the current findings.

[40] Anderson PA, Greig A, Mark TM, et al. Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing heart. Circ Res 1995;76:681-6.

[41] Babuin, L. (2005). Troponin: the biomarker of choice for the detection of cardiac injury. Canadian Medical Association Journal, 173(10), 1191–1202. doi:10.1503/cmaj/051291.

[42] Dasgupta, A., & Wahed, A. (2014). Cardiac Markers. Clinical Chemistry, Immunology and Laboratory Quality Control, 127–144. doi:10.1016/b978-0-12-407821-5.00008-5.

[43] Garg, P., Morris, P., Fazlanie, A. L., Vijayan, S., Dancso, B., Dastidar, A. G., … Haaf, P. (2017). Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. Internal and Emergency Medicine, 12(2), 147–155. doi:10.1007/s11739-017-1612-1.

[44] Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J. 2012; 33:2252–2257. doi: 10.1093/eurheartj/ehs154.

[45] Peacock, W. F., Baumann, B. M., Bruton, D., Davis, T. E., Handy, B., Jones, C. W., … Dinkel, C. (2018). Efficacy of High-Sensitivity Troponin T in Identifying Very-Low-Risk Patients with Possible Acute Coronary Syndrome. JAMA Cardiology, 3(2), 104. doi:10.1001/jamacardio.2017.4625.

[46] Kavsak, P. A., McRae, A., Vatanpour, S., Ismail, O. Z., & Worster, A. (2019). A Multicenter Assessment of the Sensitivity and Specificity for a Single High-Sensitivity Cardiac Troponin Test at Emergency Department Presentation for Hospital Admission. The Journal of Applied Laboratory Medicine, jalm.2019.029512. doi:10.1373/jalm.2019.029512.

[47] Joel A.GarciaMDJohn C.MessengerMD (2012). Myocardial Contusion. https://doi.org/10.1016/B978-1-4160-3206-9.10071-0.

[48] Ru-Yi Xu, Xiao-Fa Zhu, Ye Yang, and Ping Ye. High-sensitive cardiac troponin T. J Geriatr Cardiol. 2013 Mar; 10(1): 102–109.doi: 10.3969/j.issn.1671-5411.2013.01.015.

[49] Pettersson T, Ohlsson O, Tryding N. Increased CK-MB (mass concentration) in patients without traditional evidence of acute myocardial infarction. A risk indicator of coronary death. Eur Heart J 1992; 13:1387-92.

[50] Lee, J. J., Ahn, J., Hwang, J., Han, S. W., Lee, K. N., Kim, J. B., … Kim, E. J. (2015). Relationship between uric acid and blood pressure in different age groups. Clinical Hypertension, 21(1). doi:10.1186/s40885-015-0022-9.

[51] Topkaya, S. N., Azimzadeh, M., & Ozsoz, M. (2016). Electrochemical Biosensors for Cancer Biomarkers Detection: Recent Advances and Challenges. Electroanalysis, 28(7), 1402–1419. doi:10.1002/elan.201501174

[52] Hammond, Jules L et al. "Electrochemical biosensors and nanobiosensors." Essays in biochemistry vol. 60,1 (2016): 69-80. doi:10.1042/EBC20150008

[53] Clark Jr C, Lyons C. Electrode systems for continuous monitoring in cardiovascular surgery. Annals New York Academy of Sciences; 1962.

[54] Claussen, J.C.; Artiles, M.S.; McLamore, E.S.; Mohanty, S.; Shi, J.; Rickus, J.L.; Fisher, T.S.; Porterfield, D.M. Electrochemical glutamate biosensing with nanocube and nanosphere augmented single-walled carbon nanotube networks: A comparative study. *J. Mater. Chem.* 2011, *21*, 11224–11231.

[55] Claussen, J.C.; Hengenius, J.B.; Wickner, M.M.; Fisher, T.S.; Umulis, D.M.; Porterfield, D.M. Effects of carbon nanotube-tethered nanosphere density on amperometric biosensing: Simulation and experiment. *J. Phys. Chem. C* 2011, *115*, 20896–20904.

[56] Claussen, J.C.; Kim, S.S.; Haque, A.; Artiles, M.S.; Porterfield, D.M.; Fisher, T.S. Electrochemical glucose biosensor of platinum nanospheres connected by carbon nanotubes. *J. Diabetes Sci. Technol.* 2010, *4*, 312–319.

[57] Razia Batool, Amina Rhouati, Mian Hasnain Nawaz, Akhtar Hayat, Jean Louis Marty. A Review of the Construction of Nano-Hybrids for Electrochemical Biosensing of Glucose. *Biosensors* 2019, *9*(1), 46; doi:10.3390/bios9010046

[58] T.H. Kim, K. Abi-Samra, V. Sunkara, D.-K. Park, M. Amasia, N. Kim, J. Kim, H. Kim, M. Madou, Y.-K. Cho, Flow-enhanced electrochemical immunosensors on centrifugal microfluidic platforms, Lab Chip. 13 (2013) 3747. doi:10.1039/c3lc50374g

[59] X. Kong, X. Rao, J. Han, M. Wei, X. Duan, Layer-by-layer assembly of biprotein/layered double hydroxide ultrathin film and its electrocatalytic behavior for catechol, Biosens. Bioelectron. 26 (2010) 549–554. doi:10.1016/j.bios.2010.07.045.

[60] J.F. Rusling, G. Sotzing, F. Papadimitrakopoulosa, Designing nanomaterial-enhanced electrochemical immunosensors for cancer biomarker proteins, Bioelectrochemistry. 76 (2009) 189–194. doi:10.1016/J.BIOELECHEM.2009.03.011.

[61] Z. Cao, X. Jiang, Q. Xie, S. Yao, A third-generation hydrogen peroxide biosensor based on horseradish peroxidase immobilized in a tetrathiafulvalene tetracyanoquinodimethane/multiwalled carbon nanotubes film, Biosens. Bioelectron. 24 (2008) 222–227. doi:10.1016/j.bios.2008.03.021.

[62] Karolina Dziąbowska, Elżbieta Czaczyk and Dawid Nidzworski (December 20th 2017). Application of Electrochemical Methods in Biosensing Technologies, Biosensing Technologies for the Detection of Pathogens - A Prospective Way for Rapid Analysis, Toonika Rinken and Kairi Kivirand, IntechOpen, DOI: 10.5772/intechopen.72175.

[63] T. Bryan, X. Luo, P.R. Bueno, J.J. Davis, An optimised electrochemical biosensor for the label-free detection of C-reactive protein in blood, Biosens. Bioelectron. 39 (2013) 94–98. doi:10.1016/j.bios.2012.06.051

[64] M. Mazloum-Ardakani, L. Hosseinzadeh, Z. Taleat, Synthesis and electrocatalytic effect of Ag@Pt core-shell nanoparticles supported on reduced graphene oxide for sensitive and simple label-free electrochemical aptasensor, Biosens. Bioelectron. 74 (2015) 30– 36. doi:10.1016/j.bios.2015.05.072.

[65] S.A. Ozkan, B. Uslu, From mercury to nanosensors: Past, present and the future perspective of electrochemistry in pharmaceutical and biomedical analysis, J. Pharm. Biomed. Anal. 130 (2016) 126–140. doi:10.1016/j.jpba.2016.05.006.

[66] R.N. Goyal, S. Chatterjee, A.R.S. Rana, The effect of modifying an edge-plane pyrolytic graphite electrode with single-wall carbon nanotubes on its use for sensing diclofenac, Carbon N. Y. 48 (2010) 4136–4144. doi:10.1016/J.CARBON.2010.07.024.

[67] S.B. Revin, S.A. John, Electrochemical sensor for neurotransmitters at physiological pH using a heterocyclic conducting polymer modified electrode, Analyst. 137 (2012) 209– 215. doi:10.1039/C1AN15746A

[68] N. Karadas, B. Bozal-Palabiyik, B. Uslu, S.A. Ozkan, Functionalized carbon nanotubes - with silver nanoparticles to fabricate a sensor for the determination of zolmitriptan in its dosage forms and biological samples, Sensors Actuators, B Chem. 186 (2013) 486– 494. doi:10.1016/j.snb.2013.06.055.

[69] J. Lei, H. Ju, Nanotubes in biosensing, Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology. 2 (2010) 496–509. doi:10.1002/wnan.94

[70] P. Taylor, B. Uslu, S.A. Ozkan, Electroanalytical Application of Carbon Based Electrodes to the Pharmaceuticals Electroanalytical Application of, (2007) 37–41. doi:10.1080/00032710701242121.

[71] J.A. Ho, H.-C. Chang, N.-Y. Shih, L.-C. Wu, Y.-F. Chang, C.-C. Chen, C. Chou, Diagnostic Detection of Human Lung Cancer-Associated Antigen Using a Gold Nanoparticle-Based Electrochemical Immunosensor, Anal. Chem. 82 (2010) 5944– 5950. doi:10.1021/ac1001959.

[72] G. Ozcelikay, B. Dogan-Topal, S.A. Ozkan, An Electrochemical Sensor Based on Silver Nanoparticles-Benzalkonium Chloride for the Voltammetric Determination of Antiviral Drug Tenofovir, Electroanalysis. (2018) 1–13. doi:10.1002/elan.201700753.

[73] N. Karadas-Bakirhan, A. Sarakbi, M. Vandeput, S.A. Ozkan, J.M. Kauffmann, Liquid Chromatography with Amperometric Detection at a Silver Based Detector for the Determination of Thiocompounds: Application to the Assay of Thiopurine Antimetabolites in Urine, Anal. Chem. 87 (2015) 6730–6735.

[74] Abdulbari, H. A., & Basheer, E. A. M. (2017). *Electrochemical Biosensors: Electrode Development, Materials, Design, and Fabrication. ChemBioEng Reviews, 4(2), 92–105.* doi:10.1002/cben.201600009

[75] Yang L, Bashir R. Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. Biotechnology Advances. 2008;26:135-150. DOI: 10.1016/j.biotechadv.2007.10.003

[76] Bettazzi F, Marraza G, Minunii M. Biosensors and related bioanalytical tools. Comprehensive Analytical Chemistry. 2017;77:1-33. DOI: 10.1016/bs.coac.2017.05.003

[77] Caygill RL, Blair GE, Millner PA. A review on viral biosensors to detect human pathogens. Analytica Chimica Acta. 2010;681:8-15. DOI: 10.1016/j.aca.2010.09.038

[78] Salek-Maghsoudi A, Vakhshiteh F, Torabi R. Recent advances in biosensor technology in assessment of early diabetes biomarkers. Biosensors and Bioelectronics. 2018;99:122-135. DOI: 10.1016/j.bios.2017.07.047

[79] Karunakaran C, Bhargava K, Benjamin R, editors. Biosensors and Bioelectronics. Netherlands: Elsevier; 2015. ISBN: 978-0-12-803100-1

[80] Newman J.D., Turner A.P.F. Home blood glucose biosensors: a commercial perspective. Biosens. Bioelectron. 2005;20:2435–2453. doi: 10.1016/j.bios.2004.11.012.

[81] Tanwar H and Sachdeva R: Transdermal Drug Delivery System: A Review. Int J Pharm Sci Res 2016; 7(6): 2274-90.doi: 10.13040/IJPSR.0975-8232.7(6).2274-90

[82] V. Kalvimoorthi [1]*, M. Rajasekaran [2], V. Sundhar Rajan[3], KP. Balasubramani[3] and P. Santhosh Kumar[3]. Transdermal Drug Delivery System: An Overview. International Journal of Pharmaceutical and Chemical Sciences, 2277-500

[83] Wokovich, A., Prodduturi, S., Doub, W., Hussain, A., & Buhse, L. (2006). Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute. European Journal of Pharmaceutics and Biopharmaceutics, 64(1), 1–8. doi:10.1016/j.ejpb.2006.03.009

[84] Dipen Patel*1, Sunita A. Chaudhary1, Bhavesh Parmar1, Nikunj Bhura1. Transdermal Drug Delivery System: A Review. The Pharma Innovation (2012), 2277-7795.

[85] Wang, M., Marepally, S. K., Vemula, P. K., & Xu, C. (2016). Inorganic Nanoparticles for Transdermal Drug Delivery and Topical Application. Nanoscience in Dermatology, 57–72. doi:10.1016/b978-0-12-802926-8.00005-7

[86] Ajit Kumar Vishwakarma*, Prabhudutta Panda, Navneet Kumar Verma, Dhaneshwar Kumar Vishwakarma, Jai Narayan Mishra. An Overview on Transdermal Patches. International Journal of Pharmacy Review & Research (2017/01/01), 2248 – 9207, 2248 – 9193

[87] Prausnitz, M. R., & Langer, R. (2008). Transdermal drug delivery. Nature Biotechnology, 26(11), 1261–1268. doi:10.1038/nbt.1504

[88] Nidhi S. A Brief Review on Transdermal Patches. Organic & Medicinal Chem IJ. 2018; 7(2): 555707. doi: 10.19080/OMCIJ.2018.07.555707.

[89] Kumar, K & Kumar, Deb & Bhowmik, Debjit & Bhowmik, Jit & Chan, R & Dira, R & Dira, Dira. (2010). International Journal of Pharma and Bio Sciences V1(2)2010 TRANSDERMAL DRUG DELIVERY SYSTEM-A NOVEL DRUG DELIVERY SYSTEM AND ITS MARKET SCOPE AND OPPORTUNITIES 1 www.ijpbs.net Pharmaceutics.

[90] Bandyopadhyay A (2017) Transdermal Drug Delivery System-Quality by Design Approach. J Bioanal Biomed 9: 217-219. doi:10.4172/1948-593X.1000181

[91] Agrahari, V., Agrahari, V., Meng, J., & Mitra, A. K. (2017). Electrospun Nanofibers in Drug Delivery. Emerging Nanotechnologies for Diagnostics, Drug Delivery and Medical Devices, 189–215. doi:10.1016/b978-0-323-42978-8.00009-7

[92] Alkilani, A., McCrudden, M. T., & Donnelly, R. (2015). Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the Stratum Corneum. Pharmaceutics, 7(4), 438–470. doi:10.3390/pharmaceutics7040438

[93] Masayuki Yagi, Yoshikazu Yonei (2018). Glycative stress and skin aging. Glycative Stress Research, 2188-3610, 2188-3602

[94] Duffy K, Grossman D. The dysplastic nevus: from historical perspective to management in the modern era: part I. Historical, histologic, and clinical aspects. Journal of the American Academy of Dermatology. 2012;67(1):1. e-. e16.

[95] Dehdashtian, A., Stringer, T. P., Warren, A. J., Mu, E. W., Amirlak, B., & Shahabi, L. (2018). Anatomy and Physiology of the Skin. Melanoma, 15–26. doi:10.1007/978-3-319-78310-9_2

[96] Yousef H, Alhajj M, Sharma S. Anatomy, Skin (Integument), Epidermis. [Updated 2019 Jun 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from:__h ttps://www.ncbi.nlm.nih.gov/books/NBK470464/

[97] Slominski, A. T., Manna, P. R., & Tuckey, R. C. (2015). On the role of skin in the regulation of local and systemic steroidogenic activities. Steroids, 103, 72–88. doi: 10.1016/j.steroids.2015.04.006

[98] William D. Losquadro, MD. Anatomy of the Skin and the Pathogenesis of Nonmelanoma Skin Cancer. Facial Plast Surg Clin N Am 25 (2017) 283–289 doi.org/10.1016/j.fsc.2017.03.001

[99] Aumailley, M., & Krieg, T. (1996). Laminins: A family of diverse multifunctional molecules of basement membranes. Journal of Investigative Dermatology, 106(2), 209–214.

[100] Volz, Pierre & Boreham, Alexander & Wolf, Alexander & Kim, T. & Balke, Jens & Frombach, Janna & Hadam, Sabrina & Afraz, Zahra & Rancan, Fiorenza & Blume-Peytavi, Ulrike & Vogt, Annika & Alexiev, Ulrike. (2015). Application of Single Molecule Fluorescence Microscopy to Characterize the Penetration of a Large Amphiphilic Molecule in the Stratum Corneum of Human Skin. Int J Mol Sci. 16. 6960-77. 10.3390/ijms16046960.

[101] Parihar, Sandeep & Bhowmick, Mithun & Kumar, Rajeev & Nagar, Anand. (2013). SOFT MALLEABLE VESICLES TAILORED FOR ENHANCED DELIVERY OF ACTIVE AGENTS THROUGH THE SKIN: AN UPDATE. International journal of pharmaceutical sciences and research. 4. 172-180.

[102] Bolzinger, M.-A., Briançon, S., Pelletier, J., & Chevalier, Y. (2012). Penetration of drugs through skin, a complex rate-controlling membrane. Current Opinion in Colloid & Interface Science, 17(3), 156–165. doi: 10.1016/j.cocis.2012.02.001

[103] Ng, Keng Wooi & Lau, Wing Man. (2015). Skin Deep: The Basics of Human Skin Structure and Drug Penetration. 10.1007/978-3-662-45013-0_1.

[104] Javadzadeh, Y., & Azharshekoufeh Bahari, L. (2017). Therapeutic Nanostructures for Dermal and Transdermal Drug Delivery. Nano- and Microscale Drug Delivery Systems, 131–146. doi:10.1016/b978-0-323-52727-9.00008-x

[105] Kahraman, Emine & Güngör, Sevgi & Ozsoy, Yildiz. (2017). Potential enhancement and targeting strategies of polymeric and lipid-based nanocarriers in dermal drug delivery. Therapeutic Delivery. 8. 967-985. 10.4155/tde-2017-0075.

[106] Escobar-Chávez, José & Rodríguez Cruz, Isabel & Domínguez-Delgado, Clara & Díaz-Torres, Roberto & Revilla Vazquez, Alma & Aléncaster, Norma. (2012). Nanocarrier Systems for Transdermal Drug Delivery. 10.5772/50314.

[107] Haque, T., & Talukder, M. M. U. (2018). Chemical Enhancer: A Simplistic Way to Modulate Barrier Function of the Stratum Corneum. Advanced Pharmaceutical Bulletin, 8(2), 169–179. doi:10.15171/apb.2018.021

[108] Tejvir Kaur. Transdermal drug delivery system. Innovations in skin permeation. Innovations in Pharmaceuticals and Pharmacotherapy, 121-128, 2017, 2321–323X, 2395-0781

[109] Pawan Jalwal*1, Anju Jangra1, Lalita Dahiya1, Yashpal Sangwan2, Rajiv Saroha (2010). A review on transdermal patches. Pharma Research.0975-8216

[110] Kumar TS, Selvam RP, Singh AK. Transdermal drug delivery systems for antihypertensive drugs. Int J Pharm Biomed Res 2010; 1:1-8.

[111] Rastogi, Vaibhav & Yadav, Pragya. (2012). Transdermal drug delivery system: An overview. Asian Journal of Pharmaceutics. 6. 161. 10.4103/0973-8398.104828.

[112] Nirav S Sheth, Rajan B Mistry. Formulation and evaluation of transdermal patches and to study permeation enhancement effect of eugenol. Journal of Applied Pharmaceutical Science 01 (03); 2011: 96-101

[113] Kandavilli S, Nair V, Panchagnula R. Polymers in transdermal drug delivery systems, Pharmaceutical Technology 2002, 62-78. Available from: www.pharmtech.com. Accessed on 15 Jan, 2008

[114] Wadgave, U., & Nagesh, L. (2016). Nicotine Replacement Therapy: An Overview. International journal of health sciences, 10(3), 425–435.

[115] P. M. Patil, Dr. P. D. Chaudhari, Jalpa K Patel, K. A. Kedar, P. P. Katolkar "Recent trends in challenges and opportunities of Transdermal drug delivery system", Int. J. Drug Dev. & Res., Jan-March2012, 4(1): 39-50

[116] Todd, P. A., Goa, K. L., & Langtry, H. D. (1990). Transdermal Nitroglycerin (Glyceryl Trinitrate). Drugs, 40(6), 880–902. doi:10.2165/00003495-199040060-00009

[117] Kim, Y.-C., Park, J.-H., & Prausnitz, M. R. (2012). Microneedles for drug and vaccine delivery. Advanced Drug Delivery Reviews, 64(14), 1547–1568. doi: 10.1016/j.addr.2012.04.005

[118] Jalwal, Pawan. (2010). A review on transdermal patches. Pharma Research. 3.