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Review Article

Drugs Eluting Three Dimension Scaffolds for the Treatment of Diabetic Wounds

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ABSTRACT

Diabetes mellitus (DM) is a group of metabolic diseases identified by hyperglycemia that results from defects in insulin secretion, insulin action or both. It can also be directed as metabolism disorder. It is a disorder which affects the body's capability to generate or utilize insulin and is identified by irregular high levels of glucose in the blood.DM can lead to a number of complications such as skin, eye and foot complications, neuropathy, kidney disease, stroke, heart disease etc. Diabetic foot ulcer (DFU) is the common problem associated with those who have developed diabetes mellitus. It is one of the common and disabling and normally leads to amputation of the leg. Despite of giving treatment, ulcers tends to become chronic wounds. Although the pathophysiology of diabetic wound is multifactorial, chronic inflammation and lack of tissue regeneration leads to impair wound healing in diabetes. Scaffolds have received greater attention as they promote cell-biomaterial interactions, cell adhesion and extracellular matrix deposition and has potent advantages in treating diabetic foot ulcers. In this review, we have discussed DFU, a cellular scaffold that has been studied for DFU.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases identified by hyperglycemia that results from defects in insulin secretion, insulin action or both. Chronic hyperglycemia of DM is related with different organ failure (eyes, kidney, heart, and blood vessels), long-term damage and dysfunction also [1]. Hence, DM can also be directed as metabolism disorder [2, 3]. Metabolism means how the body is using digested food for the growth and overall development [4]. DM is a disorder which affects the body's capability to generate or utilize insulin. It is identified by irregular high levels of glucose in the blood. When the quantity of glucose into the blood increases, e.g., post meals, this provokes the discharge of the insulin from the pancreas [3]. Fig. 1 shows the different types of DM. In the development of DM many pathogenic processes are included. These ranges from autoimmune destruction of the pancreatic b-cells with consequent insulin deficiency to abnormalities, that result in resistance to insulin action.

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Diabetic foot ulcer (DFU) is the common problem associated with those who have developed DM [5]. It is a wound or an open sore that takes place in approximately 15% of diabetic patients and usually takes place in the sole of the feet. Out of all the patients who have already developed DFU, 6% are supposed to be hospitalized because of infection or any other complication in relation to ulcer. DFU usually occurs due to two reasons, first one is peripheral neuropathy and the second cause is micro and macrovascular diseases in DM [6]. In peripheral neuropathy nerve damage results in partial or complete loss of sensation in the foot or leg. Further poor blood flow due to vascular diseases might lead to development of DFU [7].

Symptoms of Diabetes and DFU

The symptoms of DM are weight loss, polydipsia, polyuria, blurred vision and polyphagia sometimes [1, 8]. The symptoms of DFU are swelling, discolouration and warmth in and around the wound. Sometimes, discharge also can be witnessed from the wound along with a foul smell. In this situation when the wound is being touched one can feel the pain and firmness.

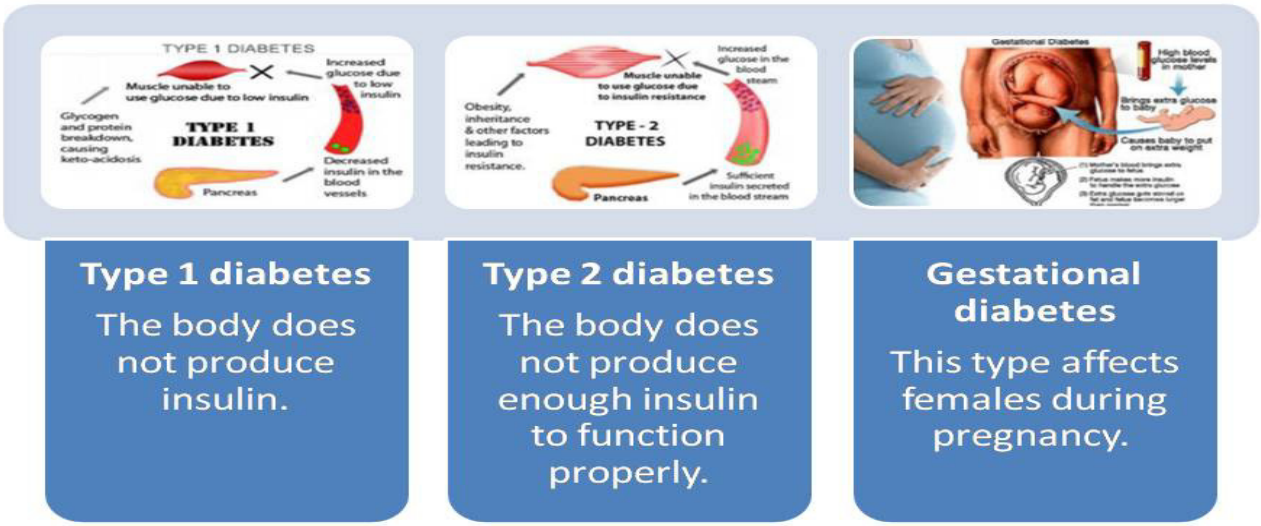

Figure 1: Types of diabetes [1]

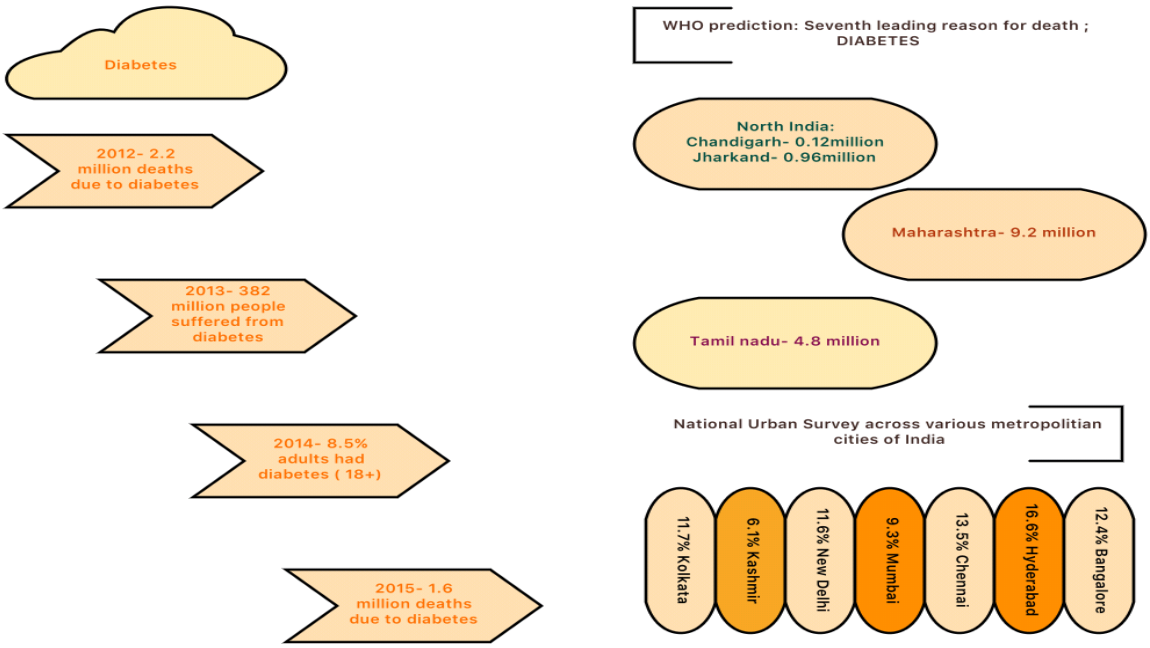

Figure 2: Data showing prevalence of diabetes (year wise), WHO survey in India depicting diabetes as a reason of death and National Urban Survey across various metropolitan cities of India

Initial things or primarily the effects that one can notice are little amount of drainage in the socks. In advanced stages of DFU, fever and chills can be noticed. Hence, it can be said that pain is no more a usual symptom. Other symptoms associated with ulceration are redness and swelling and, in some cases, when the ulceration has increased remarkably foul odour can also be noticed [9].

Epidemiology

DM is gaining the status vastly in becoming the potential epidemic in India as currently 62 million have been diagnosed with the disease [10-12]. The cases of DM are on a rise especially in middle income or low-income countries. The occurrence of DM has been predicted to be twice from 171 million in the year 2000 to 366 million in the year 2030 and the maximum boost is suspected to be in India [10, 13-14]. The disease is a major root cause of many other complications such as lower limb amputation, blindness, heart attacks, kidney failure and stroke [14]. As per the survey conducted in United States, 15% of diabetic patients develop DFU in their lifetime and around 80,000 amputations have been reported per year [15]. Fig. 2, showing WHO survey and National Urban Survey conducted across India.

Pathophysiology of DM and DFU

The term 'diabetes' is obtained from the ancient Greek word 'diabainen', which means 'go through', to indicate the extreme passing of urine via kidney. Later the term Mellitus was added to it which means 'sweet' in order to distinguish it from an excessive production of non-sweet urine which is Diabetes 'insipidus' [16, 17]. Now, it is quite acknowledged that DM is an autoimmune disorder marked by the devastation of insulin-producing pancreatic β-cells. DM shows heterogeneity like many other immune-mediated diseases, in terms of age of onset, severity of autoimmune response and efficacy of therapy [16, 18]. There are many serious complications related with the DM and DFU is among the serious one. The reasons why diabetes leads to this serious stage are peripheral neuropathy, immunosuppression and peripheral arterial disease [15]. In figure 3, it has been clearly stated that how hyperglycemia can lead to amputation.

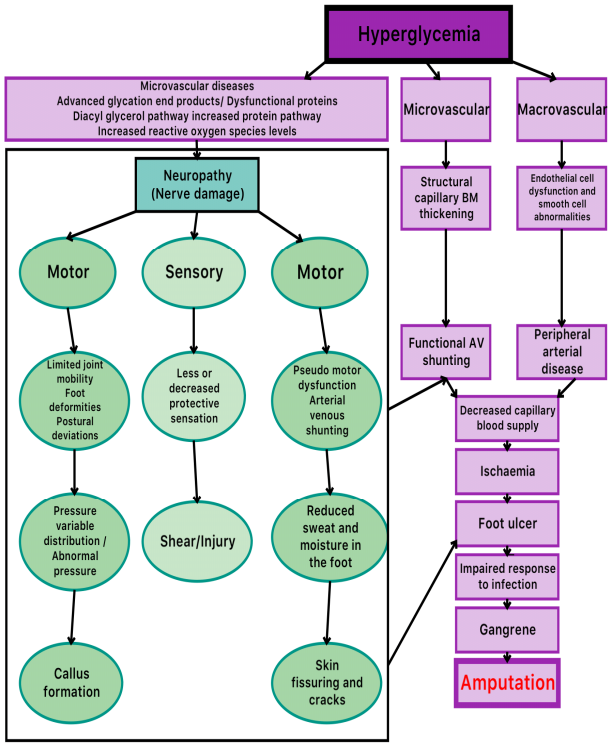

Figure 3: Picture showing how hyperglycemia leads to amputation

Treatments for DM and DFU

The basic and generally opted treatments for the treatment of DM are diet which includes balanced and nutritious diet, workouts, medications and sometimes insulin therapy is also required. Some of the drugs for the treatment of DM has been mentioned in the table 1.For the successful treatment of DFU many factors are present to stick to such as preventing the infection, offloading (not to pressurize the area), debridement (removal of dead tissue and skin), application of suitable dressing and medications on the ulcerated area, management of blood glucose levels and lastly other problems related to health [9].

Table 1: The drugs that can be used for treating Diabetes along with their actions [19-21]

Drugs	Action
Chlorpropamide, Glimepiride, Glipizide, Glyburide, Nateglinide and Repaglinide.	Increasing Insulin production
Metformin	Decreasing Insulin production
Acarbose and Miglitol	Decreasing sugar absorption
Pioglitazone and Rosiglitazone	Improving body insulin
Canaglifozin, Dapagliflozin, and Empagliflozin	Blocking the reabsorption of glucose by kidney & increasing glucose excretion in urine
Pramlinitide	Decreasing blood sugar after meals in diabetics who are on insulin
Afrezza	Rapid-acting inhaled insulin
Tresiba	Name is Insulin degludec, ultralong-acting basal insulin

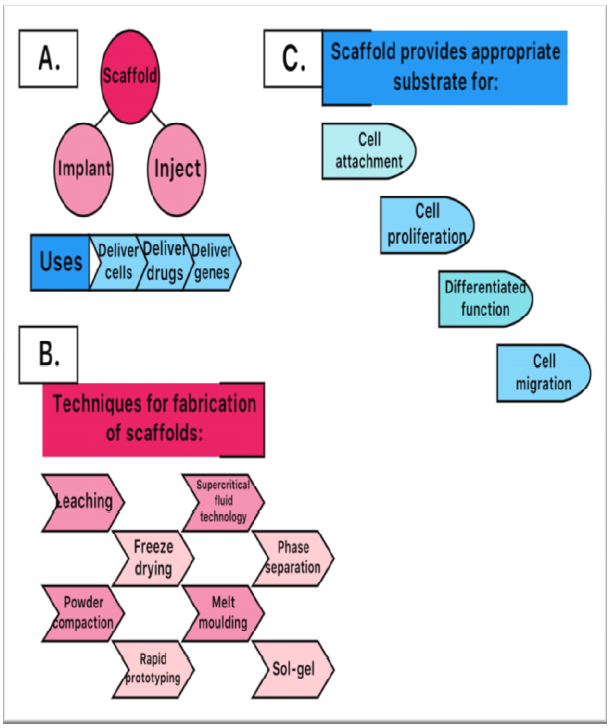

Figure 4: Pictorial representation of Scaffolds. a) Types of scaffold b) Techniques for fabrication of scaffolds c) Scaffold provide appropriate substrate for cell attachment, cell proliferation. Differentiated function and cell migration.

Scaffold: A Novel Carrier for Cell and Drug Delivery [22]

Tissue engineering focuses to restore or ease the re-growth of damaged or diseased tissue by applying a combination of biomaterials, cells or bioactive molecules [23]. Fig. 4 and 5 demonstrates briefly about scaffolds. Figure 6illustrates the properties of scaffold. The damaged or diseased tissue isreplaced by using donor graft tissues (autografts, allografts, or xenografts) but the disadvantages linked with this are as follows [24]:

- shortage of donors or donor sites
- transmission of disease
- rejection of grafts
- donor site pain and morbidity
- the volume of donor tissue that can be safely harvested and
- The possibility of harmful immune responses [24].

Biodegradable polymeric scaffolds for tissue engineering have attained enormous attention because they impart a temporal and spatial environment for cellular growth and tissue in-growth. Scaffold is the chief component that is employed to incorporate cells, drugs, and genes into the body [25]. There are three systems by that scaffolds can be used, first one goes like space filling system, also called as 'conductive'. In this type of system, a framework is being provided for the tissue generation. The other uses of systems are as bioactive molecule delivery system, also called as 'inductive' and the last use is as cell/tissue delivery system, also known as 'cell seeding' [26-28].

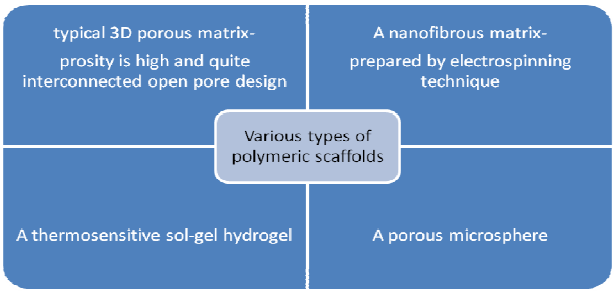

Figure 5: Different types of scaffolds

Tissue engineering technologies involve the proper and successful interaction between three components:

- the scaffold that holds the cells together to create the tissue's physical form;
- the cells that create the tissue; and
- The biological signalling molecules, such as growth factors, that direct the cells to express the desired tissue phenotype [29].

Scaffold for tissue engineering (cell delivery) should possess the following [29]:

- Mechanical properties that are sufficient to shield cells from tensile forces without inhibiting biomechanical cues [29]
- Desired volume, shape, and mechanical strength
- Acceptable biocompatibility
- A highly porous and well-interconnected open pore structure to allow high cell seeding density and tissue in-growth
- Bio-adsorption at predetermined time period
- Biocompatible chemical compositions and their degradation products, causing minimal immune or inflammatory responses
- Physical structure to support cell adhesion and proliferation, facilitating cell-cell contact and cell migration

Following are the properties a Scaffold shoul exhibit to become a drug delivery system:

- Homogenous drug dispersion throughout the scaffold [30]
- Ability to release the drug at a predetermined rate [30]
- Drug binding affinity that is sufficiently low to allow the drug released to be stable when incorporated in the scaffold at a physiological temperature [31]
- Stable physical dimension, chemical structure, and biological activity over a prolonged period of time [31].

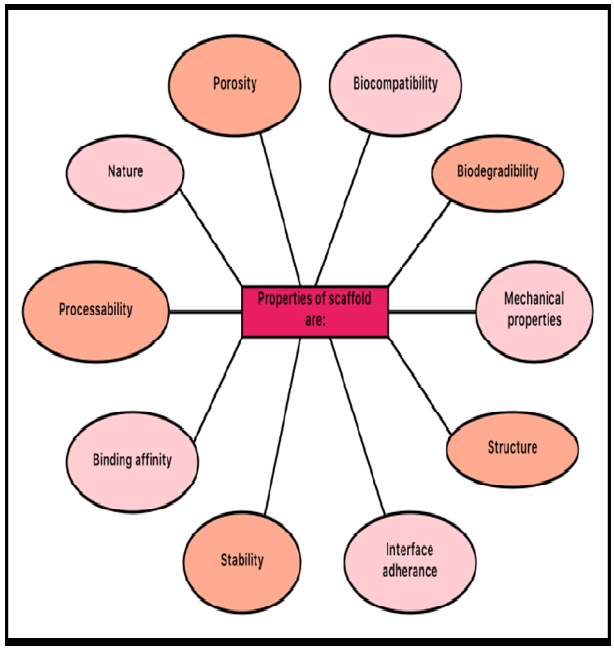

Figure 7: 'Properties of scaffold'

Characterization of Scaffolds

- Scanning electron microscope (SEM) studies shows the porosity of the scaffolds that has been developed for the diabetic wound.
- Fourier transform infrared studies will be predicting about the chemical stability and integrity of the scaffold developed.
- Enzymatic degradation and MTT assay will be helping in establishing the reabsorption and non-cytotoxicity of the developed scaffold.
- The Enzymatic degradation will be helping in suggesting about the weight loss during incubation.
- Cytotoxic analysis will be determining the noncytotoxic behaviour of scaffolds towards the normal human dermal fibroblast cell lines.
- Scanning electron microscopy analysis will be conforming about the about the cellular adhesion of the scaffold with the normal cells.
- In vivo studies will be conducted using excision and incision wound models in rodents.
- Histological examination will be done to demonstrate the re-epithelialization in the wound.

Biomaterials for Scaffold Fabrication: Polymers are synthesized by joining small molecules into a single giant molecule by a chemical process. The small molecules which are used in the synthesisof a polymer are known as monomer. Polymers are found broadly in life [32].

Natural Polymers

Natural Polymers are those substances which are obtained naturally. These polymers are formed either by the process of addition polymerization or condensation polymerization [32]. The advantages of them are biocompatibility, commercial availability, easy processing and they more closely mimic the natural extracellular matrix of tissues [33]. However, they possess some limitations also such as short supply, expense, batch to batch variation and susceptibility to cross-contamination [33].

Examples of natural polymers are alginate, proteins, collagens, gelatin, fibrins, albumin, elsinan, pectin (pectinic acid), galactan, curdlan, gellan, levan, emulsan, dextran, pullulan, gluten, elastin, fibroin, hyaluronic acid, cellulose, starch, chitosan (chitin), scleroglucan, heparin, silk, chondroitin 6-sulfate, and polyhydroxyalkanoates [33].

Synthetic Polymers

Synthetic Polymers are derived synthetically through chemical processes.

They are of two types Biodegradable-polyglycolide, polylactide and its copolymer poly(lactide-co-glycolide), polyphosphazene, polyanhydride, poly (propylene fumarate), polycyanoacrylate, polycaprolactone, polydioxanone, and polyurethanes. Non-biodegradable-polyvinyl alcohol, polyhydroxyethymethacrylate, and poly(N-isopropylacrylamide [33].

Following are the drugs that have been incorporated successfully in the scaffolds for treating the DFU

In the year 2008, the author Shu-Hua Teng et.al. has used Chitosan and Hydroxyapatite (HA) in preparation of scaffolds and tetracycline hydrochloride (TCH) have been used as a drug in the preparation. The very first step done by them is to prepare a porous composite scaffold and TCH is impregnated in the scaffold as a model drug. The pore size of the scaffold is found to be negatively dependent on the HA content and ranged about 40–250 μm. Subsequently, a porous chitosan /HA composite layer without drug is coated on the scaffold in order to create a gradient drug concentration in the specimen**.** The in vitro drug-release test conducted by them demonstrated that the porous layer without drug on the outer surface of the scaffold significantly reduced the initial burst of drug release and release period is found to be extended. Finally, a successive and dense chitosan /HA composite layer endowed the scaffold with a sustained, drug release pattern without any initial drug burst. These findings by the author confirmed the high effectiveness of the hybrid scaffolds in regulating the release of drugs, and hence their capability to serve as a temporary drug carrier in tissue regeneration. These functional scaffolds also have potential application to the delivery of some bioactive molecules such as growth factors since, it is a novel technique so the techniques used in here are co-precipitation, moulding and freeze drying. This study has shown that by varying the HA concentrations the drug release kinetics of TCH can also be controlled [34].

In the year 2008 Erhan Pis kin *et. al*., have designed simvastatin loaded scaffold successfully, another approach in the field of

tissue engineering. Here, the method used is electro spinning which is attracting a lot of attraction in tissue engineering. Currently cellular scaffolds containing bioactive agents have been used by them for the soft and hard tissue repair. For the simulation of bone formation simvastatin, an active drug is loaded either after the membranes formed or during the electro spinning. In rats cranial defects are created, 8mm in diameter and then the simvastatin loaded scaffold is applied to the defect. Samples are taken after 1,3 and 6 months of implantation. Bone regeneration, tissue response, X-ray micro-computed tomography and histological analysis are some of the tests performed by them. The loading of drug into the scaffold was accomplished by two methods, the first one being is drug solution added into the scaffold whereas the other one is calculated dose of drug was dispersed into the PCL solution. So, here it is being called as 'Simvastatin loaded PCL scaffold'. Here the loading of drug suggests that the drug release will be slow, long-term and degradation will be in a controlled manner [35].

In the year 2009, author N. Adhiraj et.al. added Doxycyclineinto microspheres and then resurfaced into the collagen scaffolds. This drug being broad-spectrum antibiotic and is quite productive against gram-positive, gram negative, protozoa and various anaerobes. In order to penetrate deep into the infection with effective and efficacious drug concentration and to bypass side effects, controlled local delivery of Doxycycline is required. The major benefit of this drug loaded scaffold is that it releases the drug in a slow manner and thus it reduces the frequency of changing the dressing as compared to other dosage forms, which requires proper monitoring [36].

In the year 2016, author Veera Venkata Satyanarayana Reddy Karri et.al. have used curcumin (CUR) into the scaffolds for the treatment of diabetic wound healing. CUR is known as anti-oxidant and anti-inflammatory agent. Since it is poorly water-soluble and stability issues are also there, so it's been added into first chitosan nanoparticles (CSNSPs) and later CUR-NSPs in collagen scaffold. The method of preparation of scaffold used by them here is Freeze drying. For evaluation purpose methods used are particle size, zeta potential, SEM, differential scanning calorimetry and X-ray powder diffraction. The prepared formulation provides some prominent properties like biocompatibility, anti-inflammatory, proliferation and cell adhesion which are essential for tissue engineering in impeding wounds of diabetes [5].

In the year 2012, Devasier Bennet et.al. have incorporated Quercetin (an antioxidant) and Voglibose (hypoglycemic) into scaffolds. The incorporation of dual drug loaded nanoparticles into the scaffold film is achieved by the technique solvent casting method thus in turn created a controlled transdermal drug delivery system. The prepared scaffold film demonstrated superior capability of encapsulation and swelling characteristics and many other potential uses (example in various diabetes-related complications). The characterization includes morphology by Field emission scanning electron microscopy and size distribution by Photon correlation spectroscopy, particle size reduction by Malvern zetasizer, drug entrapment efficiency etc. This scaffold film formulation is a new type of hybrid-integrated film and it also possesses some distinctive qualities for the transdermal drug delivery system [15].

In the year 2012, Sangeeta Kumari et.al. have prepared Chitosan-g-glycolic acid and Au-Fe_3O_4 hybrid nanoparticles and dispersed in glycolic acid grafted chitosan scaffolds. The method of preparation followed here is vacuum and freeze drying. For characterization of the prepared scaffold they followed the techniques as follows; Transmission electron microscopy, Physical property measuring system, attenuated total reflectance Fourier transform infrared spectroscopy, scanning electron microscopy, Swelling behaviour, In vitro drug release and cell culture study and cell proliferation assay. The prepared scaffold poses porous morphology. The scaffolds prepared are found to be stable irrespective of the pH of the medium. These have also shown higher and faster drug release. In the end, they have concluded by saying that the prepared scaffold offers great advantage over others and properties that are crucial for the tissue engineering purpose [9].

In the year 2013, Maurice N. Collins and Colin Birkinshaw have used Hyaluronic acid as tissue scaffolding material. Their review has focused on the introduction of scaffold followed by their mechanism of action. Fundamentals and Hydrodynamics of Hyaluronic acid has been illustrated well by them. Tissue engineering applications has also been discussed widely

along with some prime characteristics of the scaffolds such as mechanical, biological function and degradation have also been conveyed. This review focused on the latest technologies regarding to scaffold preparation for tissue engineering [26].

In the year 2014, Cheng-Hung Li et.al. have developed drug-eluting membranes (biodegradable nanofibrous) using metformin for the sustained release of the drug in case of treating the wounds caused by DM. Here in order to prepare the biodegradable membranes the technique used for the preparation is electro spinning. For the characterization purpose the methods used are elution method and HPLC assay, carried out to find out the in vivo and in vitro release rate of the active pharmaceutical ingredient from the membrane prepared. In the end of the study the conclusion came out to be that metformin loaded membranes released the high concentrations of drug for more than three weeks [27].

CONCLUSION

In this article we have mentioned about DM and DFU, it's symptoms, appearance of DFU, epidemiology and pathophysiology. The drugs which are widely available in market for the treatment of DM has been given. A detailed description about scaffolds for the treatment of DFU has been given, along with its method of preparation, types, ideal properties and polymers to be used for the preparation of scaffolds. Characterization of scaffolds has also been discussed. The drugs incorporated into scaffolds by far has also been discussed.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest involved inthis study. The authors alone are responsible for thecontent and writing of the paper.

AUTHOR'S CONTRIBUTION

Rishita Tyagiis the lead author and synthesized the literature Veera Venkata Satyanarayana Reddy Karri provided design and conceptual input, Bharat Kumar Reddy Sanapalli helped in drafting and critical revision of the manuscript. All authors read and approved the final paper.

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