Dendrimer based nanoarchitectures in diabetes management: An overview

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ABSTRACT

Diabetes has turned out to be one of the biggest worldwide health and economic burdens, with its expanded predominance and high complexity proportion. The quantity of diabetic patients is expanding enormously around the world. Several reports have demonstrated the sharp increment in the sufferers. Stable and acceptable blood glucose control is fundamental to diminish diabetes-related complications. Consequently, ceaseless endeavors have been made in antidiabetic drugs, treatment strategies, and nanotechnology based products to accomplish better diabetes control. The nanocarriers pertaining hypoglycaemics provide improved diabetes management with minimum risk of associated side effects. Dendrimers have caught an incredible attention in the field of drug delivery and personalized medicines. Dendrimers are three-dimensional well-defined homogenous nanosized structures consisting tree-like branches. The present review highlights the different aspects of dendrimers including fabrication, surface engineering, toxicological profile as well as delivery of antidiabetic drugs for the effective cure of diabetes.

Keywords: Diabetes, Dendrimer, Drug delivery, Toxicity, Nanocarrier

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that affects billions of people all over the world [1, 2]. It is one of the most prevalent life-threatening metabolic disorders, included amid top 3 non-transmissible disorders, which accounts for more than 80% of non-transmissible ailments related deaths and superlative 10 reasons of death around the world. Its worldwide occurrence has quickly expanded in recent decades [3]. As per International Diabetes Federation (IDF) estimation, more than 96,000 new instances in adolescents and children (less than 15 years age) of Type-1 diabetes were identified and analyzed globally each year since 2017. The nations with top 10 most noteworthy weight by number are the United Kingdom, United States of America, Brazil, India, China, Germany, Russian Federation, Nigeria, Algeria and Saudi Arabia, representing about 60% of entire new cases.

The childrens' case of Type-1 diabetes differs about 400-fold among countries, rates of age-adjusted case ranges from 0.1 per 100,000 every year in countries such as China and Venezuela, up to 37.8 in Italy (especially Sardinia) and 40.9 per 100,000/year in Finland. In general, North America and Europe have higher rate, as the incidences are comparatively low in Africa and Asia respectively, with the Kuwait remarkable exception [4].

"Diabetes" (means a "siphon" or "to go through" which refers to the chronic polyuria, one of the prominent characteristics of diabetes) name was conceived from the Greco-Roman doctor, Aretaeus of Cappadoccia amid of 30-90 Common Era (CE). The earliest diabetes mellitus description originates from the Ebers Papyrus, believed that Egyptian doctor Hesi Ra has written about the disease around 1550 Before Common Era (BCE). Indian physicians developed the earliest recorded clinical sample for DM. Susruta, the God of Ayurvedic medicines, precisely described diabetes in 500-600 BCE. In the subsequent century, Charaka was the first well-known

Ayurvedic specialist, recognized the contrast between two types of diabetics. In modern medicine the two types are referred as Type-1 DM (T1DM) and Type-2 DM (T2DM) [5, 6].

Since Banting, MacLeod and Best discovered insulin (a peptide hormone with 51 amino acids framing 2 chains A and B) in 1921, no further effectual drug that can/may supplant insulin have been discovered. Modification of insulin structure during processing, storage and administration presents a challenge in insulin storage and diabetes treatment [7-10]. As per International Diabetes Federation (IDF) data, about 451 million adult individuals had diabetes in 2017 and it is expected to increase to about 693 million in 2045. The number of adolescents and children suffering from T1DM (0-19 years) is anticipated to be 1,106,500 around the world. The increased T1DM incidence in young age group accounted as 2.4% [11].

The hyperglycemia (constant) results in interminable macro- and micro-vascular effects, for example, cardiovascular complications, nephropathy, neuropathy, retinopathy, and stroke [12]. Chronic hyperglycemia (elevated blood sugar level) is related with damage and failure of different organ systems, affecting nerves, eyes, heart, kidneys, and may eventually develop nephropathies, cerebrovascular infections, neuropathies, ischemic heart sicknesses, retinopathies, diabetic foot ulcer and fringe vascular ailments [13-16]. The drugs used in the management of hyperglycemic situation in diabetes are classified into two groups based on the route of administration, (such as oral hypoglycemics and parenteral insulin) and glucagon-like peptide-1 (GLP-1) receptor agonists [12].

2. PATHOPHYSIOLOGY OF DIABETES MELLITUS

2.1. Type-1 diabetes mellitus

Type-1 diabetes mellitus (T1DM) is an insulin-dependent diabetes, resulted from the deficiency of insulin due to the progressive deterioration of the pancreatic β-cells by an autoimmune

reaction. There are many factors that can either generate or are associated with reaction of autoimmune system are, viruses, genetics, dairy animals' milk, and reactive oxygen species or free radicals. Histological studies performed on the pancreatic cells of patients with T1DM, indicated infiltration of different immunity cells like B and T lymphocytes, dendritic cells, macrophages, natural executioner cells, just as islet-responsive antibodies and T-lymphocytes in the Langerhans' islets [17]. The pathogenesis of T1DM is based on the β-cells damage or turnover, leading to the release of autoantigens. Thus, β-cells autoimmune-antigens are introduced through antigen-presenting cells (APC) to the helper T-cells. In combination of major histocompatibility complex (MHC), APC will relocate to the lymph node of the pancreas. Autoreactive and autoantibodies T-lymphocyte cells activates in the presence of APC and coordinated in contrast to auto-antigens of β-cells [18]. The activated T-cells then encounter related β-cell antigens further reactivated once again, consequently killing the β-cells. T-cells and macrophages forms cytokines (such as interleukin-22 (IL-22), tumor necrosis factor-β (TNF-β), and interferon-α (IFN-α)) that results in the improvement of T1DM symptoms [19]. An inflammatory reaction may also be induced due to nitric oxide synthase (NOS) generation, and development of reactive oxygen species (ROS).

The reactive oxygen species (ROS) damages β-cells which results in tissue devastation [20, 21]. Other than that, the apoptosis-inducing receptors expression, cytokines can activate Fas or CD95L-a ligand, type II transmembrane protein belonging to TNF, ultimately result in Fas-mediated β-cells apoptosis. Likewise, β-cells destroyed when Fas surface ligands came in contact of an effector T-lymphocytes. These effector T-lymphocytes facilitates the protease granzymes passage via secretion of molecules of perforin (a pore forming cytolytic protein found in the granules of cytotoxic T lymphocytes and natural killer cells) [17]. Granzymes destroys these β-

cells via permitting the pathway of perforin molecules and activates endogenous nucleases. Macrophages generate miscible mediator, for example, cytokines and ROS like interferon gamma (IFN-γ) and interleukin-1 beta (IL-1β) through lipopolysaccharides and tumor necrosis factor-alpha (TNF-α) stimulation and further damages β-cell, which eventually leads to self-destruction once come in contact with specific environment [20, 21]. During inflammation, β-cells increment the MHC-II quantity and thus show antigen to the diabetogenic CD4 T-lymphocytes [20]. Furthermore, numerous chemokines developed in β-cells infiltrates the Langerhans islets and employ immune cells to pancreas by means of chemokine receptors [22]. Both, natural and versatile immunity contribute to the development of T1DM.

2.2. Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is fundamentally connected with the insulin resistance. Instead of solitary "diabetic gene," there seems to be lack of genetic defense that incorporates errors on some genes. The estimated post-insulin receptor intracellular errors results in hyper-insulinemia and further insulin resistance. Furthermore, T2DM consists of an impaired insulin secretary reaction to glucose and diminished insulin adequacy in stimulating glucose take-up through skeletal muscles, also in controlling the hepatic production of glucose. This hyperinsulinemia is significantly ascribed to obesity (mainly stomach obesity), coronary artery diseases/ailment (CAD), hypertension and dyslipidemia [23]. This group of five abnormalities is considered as resistance of insulin syndrome, disorder X or Kaplan's disorder. Other factors in the T2DM development are age, obesity, and the lifestyle. Chronic viral infections such as [universal herpes-type infections, for example, cytomegalovirus infection (CMV), human herpes virus six (HHV6) and human herpes virus seven (HHV7)] may sometime also have an influence in initiating T2DM. These types of viruses may stay dormant inside the body for several years or

decades, however they may turn active later due to maturing, sickness, stress, or poor diet routine [5]. Ongoing examination of 2 markers of C-responsive protein, systemic inflammation and IL-6, proposes that T2DM could be related with systemic inflammation [24]. It is also demonstrated that adults, who gets less than 6.5 hr of sleep/night have a 40% lower insulin affectability than adults who get full eight hours of sleep/night. Curtailment in sleep in young adults weakens the insulin efficiency to do its work efficiently and can cause hypertension, unusual lipid levels, and weight gain [25]. The insulin sensitivity fluctuates with the uptake of starch rich foods, measure of physical action and stress signals. A high fatty acid state with hyperglycaemic conditions can prompt to decrease the insulin gene expression [26]. Impaired functioning of cholesterol transporters can also destroy the β-cells via accumulation of sterol and inflammation in islets cells [27]. Fraction of lipoprotein and metabolism of cholesterol leads to the failure of β-cell function. The low-density lipoprotein (LDL) experiencing oxidization can diminish the pre-proinsulin expression in isolated β-cells, while extremely LDL can induce apoptosis of the β-cells. Conversely, there are reports that analyzed the results uncovering the defensive actions of high-density lipoproteisn (HDL) on β-cells. They influence valuably on the glucose homeostasis by increasing the function of pancreatic β-cells and disposal of plasma glucose [28].

Lipid interacts with incretin hormones, especially GLP-1, via down regulating their receptors and adjusting the downstream cyclic adenosine monophosphate (cAMP) signaling, along with the negative influence on incretin signaling which advances the secretion of insulin and survival of β-cells [29]. In addition, unsaturated fats can potentiate the inflammatory toxicity via activating specific inflammatory pathways, as fat tissues are essential in generating inflammatory mediators, such as TNF-α, Monocyte chemoattractant protein-1 (MCP-1), Plasminogen activator inhibitor (PAI-1), Interleukin-6 (IL-6) and IL-1β [30]. Inflammatory mediators enhance the

immunity cells and inflammatory cytokines production, which results in inflammation of islets in the T2DM pancreas *in vivo*. In *in vitro* studies a high fat diet feeding, generation of islets macrophage migration inhibitory factor (MIF) was expanded which directs β-cells to the lipotoxic cell apoptosis [31]. Lipids can also influence the sensitivity of insulin by mounting the intracellular di-acylglycerols accumulation; a triacylglycerol compound that shows a negative result on signaling of insulin. Moreover, lipids improve protein kinase C proteins, play their role in restraining signaling of insulin transduction [32]. Saturated fatty acids (SFAs) activates Toll-like receptor 4 (TLR4), which consecutively mediated the insulin resistance-related inflammation by up-regulating the expression of NF-κB transcription factors, IκB kinase (IKK) and pro-inflammatory mediators in fat tissue macrophages [33]. However, it can be deduced that inflammation is involved and engaged in the pathogenesis of T1DM and T2DM (**Fig. 1**).

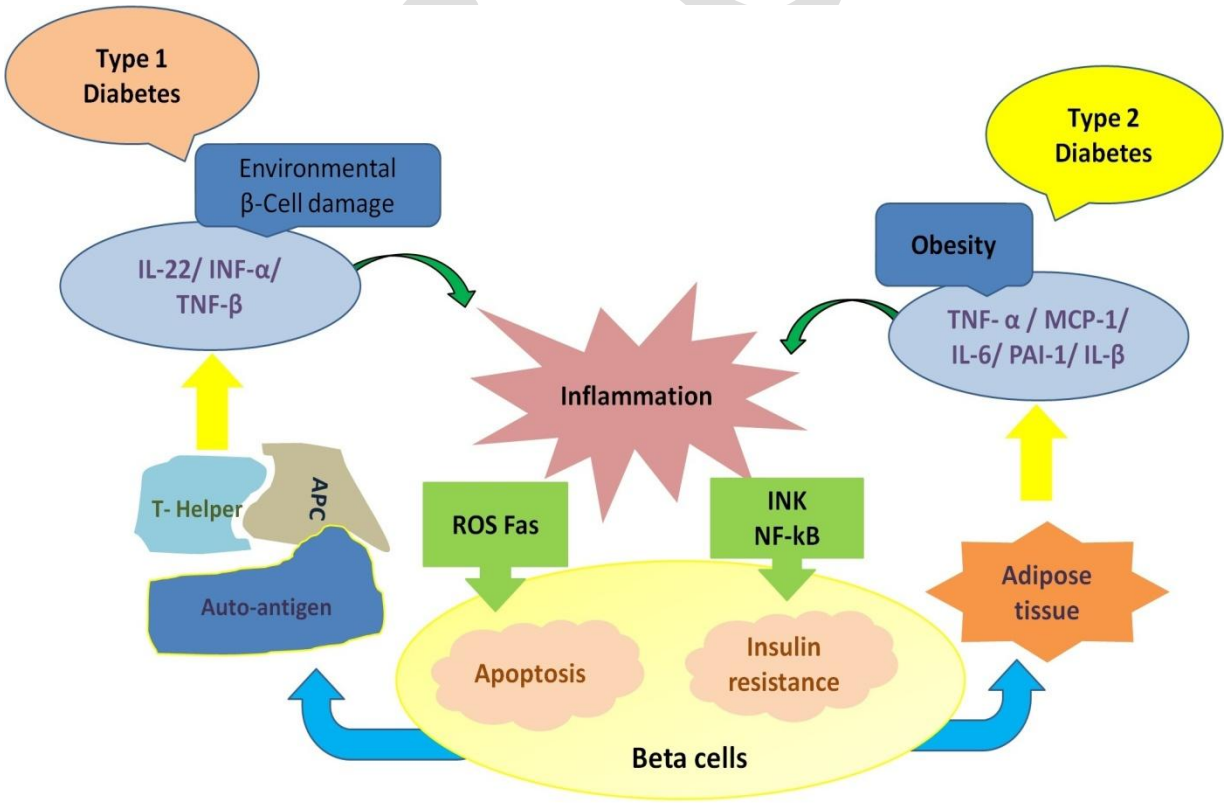


Fig. 1. Role of inflammation in pathogenesis of T1DM and T2DM

3. TYPES OF DIABETES MELLITUS

It is widely acknowledged that there are three fundamental classes of diabetes, *i.e.*T1DM, T2DM and gestational diabetes (GDM). T2DM establishes 90% of all diabetes cases [34].

3.1. Type 1 diabetes

T1DM is also known and refereed as juvenile-onset diabetes, juvenile diabetes, ketosis-prone diabetes, brittle diabetes, insulin-dependent or immune-mediated. It is brought about by an auto-immune response, where the body's immune system attacks insulin-generating cells. Individuals with T1DM produces no or negligible amount of insulin. The disorder can influence people of all age group; however, it is more often observed in young adults or children [35]. Insulin treatment is required for T1DM, despite the fact that the disease may develop at any age, it usually attacks adolescence or childhood and the prevalent diabetes type analyzed before the age of 30. Exemplary indications of T1DM include: more thirst (polydipsia), more urination (polyuria), increased hunger, rapid weight loss, vision changes and fatigue. If T1DM left unmanaged, people can succumb to diabetic keto acidosis, which may lead to death or coma [5].

Recent development in the area of role of microbiota uncovers the emergence of gut microbiota as the main source in the progression of an autoimmune disorder, including T1DM. Mutually with the intrinsic immunity, gut microbiota acted on path recognition receptors (PRR) and directs the native inflammatory reaction. The PRR perceives pathogen-associated molecular pattern (PAMPs) and promote the induction of native immune reaction leading to pro-inflammatory cytokines, advances autophagy, interferons and phagocytosis activity that encourages the eventual cell death. For example, toll-like receptors (TLR) 7, TLR-8, Myeloid differentiation primary response protein (MyD88), and NLPR3 gene are responsible for T1DM predisposing [36].

A strong correlation between T1DM analyzed before the age of 30 and explicit HLA-D (human leukocyte antigen type D) phenotypes such as HLA-DR3/DR4, HLA-DR3 and HLA-DR4 was observed in Caucasian population [37]. Of individuals recently diagnosed with T1DM, 70-80% consists of antibodies for their islet cells, 30-50% with antibodies for insulin, and 80-95% comprises antibodies for glutamic acid decarboxylase (GAD), which is an enzyme required by beta cells of pancreas to function efficiently [38]. Infection caused by Coxsackie B4 virus play its role in the T1DM development by provoking the autoantibodies generation targeting GAD, as a small region of GAD molecule is almost identical to a protein region found on the virus. . Studies in diabetes-inclined rodents demonstrate that retaining soy and wheat may help to prevent or delay the onset of diabetes [33]. The O_2 free radicals developed as a result of the numerous chemical and metabolic reactions in body, destroys own body cells and islet cells, which have low enzyme level that can separate the free radicals. In this way, factors that can induce the endogenous production of free radicals (such as, air contamination, unhealth dietary habits, and smoking) may damage pancreatic cells. Likewise, there are many chemicals agents that can contribute towards the development of T1DM, for example, pyriminil, a rodent toxin, and two physicians endorsed drugs, L-asparaginase and pentamidine.

3.2. Type 2 Diabetes

T2DM is determined by insulin resistance and decrease of insulin production [6]. In T2DM or non-insulin dependent diabetes (NIDDM), the pancreas still forms the insulin. The concern is unresponsiveness of the insulin receptors which eventually causes inefficient hepatic metabolism of glucose. This is alluded to as insulin resistance. In this situation, pancreas produces more insulin to regulate high blood glucose level. Although, the cells are not able to respond, therefore the blood glucose level remains high. After some time, this elevated level of blood glucose starts

to harm the body by the accumulation of glycation and sorbitol proteins, causing indications like exhaustion, nocturia, general malaise, consistent thirst, unexpected weight reduction, vision changes (blurring or poor centering), diminished immunity, slow mending capacity from wounds or cuts. When this remains untreated, the damage caused by T2DM become irreversible, prompting perpetual health issues, for example, renal disappointment, visual deficiency, and vascular trade off.

T2DM is described as a dynamic decrease in pancreatic β-cell activity and expanded insulin obstruction, and records for around 90% of individuals with DM [40]. T2DM have seen highest increase in prevalence, generally determined by lifestyle incorporating changes in dietary habit and patterns, declining physical movement level, and expanding sedentary practices [41]. T2DM is also known as ketosis-resistance diabetes and maturity onset diabetes (MOD) [5]. This is the most widely recognized type of diabetes analyzed in individuals who are 30 years of age or older. It might happen in children or adolescents and alluded to as maturity onset diabetes in young (MODY). Although, most of the patients were managed with exercise, eating healthy diet, and oral medications, some patients may persistently or intermittently needs insulin to regulate the symptomatic hyperglycemia and avoid non-ketotic hyperglycemic hyperosmolar coma (NKHHC) [37].

3.3. Gestational diabetes

Gestational diabetes (GDM) referred to diabetic conditions analyzed only during the gestation period or pregnancy. Gestational diabetes is observed in 2-5% cases of all pregnancies. Despite the fact that this type of diabetes may immediately remit after the childbirth, if ignored or left untreated, it may cause stillbirth, macrosomia, fetal respiratory distress and birth defects. Likewise, it may incline both infant and mother to developT2DM later on [5]. One out of six live

births happen in women with DM, of which the most general type accounts for around 87.5% of all diabetes during pregnancy is GDM. Offspring risks include high adiposity, birthweight and macrosomia that is large for gestational age (LGA). Though, severe perinatal difficulties (for example, shoulder dystocia, death, fracture or nerve paralysis) are uncommon (1-4%), macrosomia (newborn child birth weight >4 kg) and large for gestational age (birth weight >90%) are normal, influencing 10-20% of neonates birth to women with GDM [42].

The hereditary premise of T2DM is unknown yet; several further types have been genetically analyzed. The most widely recognized type is MODY, a familial type of diabetes acquired in an autosomal predominant way and related with mutations in certain hepatic genes or β-cell (for example glucokinase and HNF homeobox A (HNF-1a)). Further, a well-described type of diabetes (secondary DM) involve diabetes related with pancreatic infection (for example haemochromatosis-associated diabetes), corticosteroid (or other) hormone extremes or definite drug (for example, protease inhibitors in human immunodeficiency virus (HIV) infection, immune checkpoint inhibitors in tumor treatment, and a typical antipsychotic drug in schizophrenia management) [43].

4. DENDRIMERS AS FUTURE SMART DRUG CARRIERS FOR THE MANAGEMENT OF DIABETES MELLITUS

4.1. Dendrimers

Dendrimers are homogenous well-defined 3D structure having tree-like branches [44, 45]. Dendrimers have caught an extraordinary attention in the drug delivery field to achieve controlled drug delivery and in development of modified medicine systems [46]. In 1978, Vogtle et al. made the first endeavor to design and develop the dendritic framework [47]. These molecules were primarily referred as "cascade molecules". Tomalia et al, established another

class of cascade molecules with amides, with small structures [48]. They refereed these innovative dendritic macromolecules as "dendrimers". The name dendrimer is derived from the Greek words "dendros", signifies "branch or tree" and "meros" means "part" [49]. Meanwhile, Newkome and co-workers studied synthesis of analogous macromolecules and named "arborols", based on “arbor”, which is a Latin word for tree [50].

Dendrimers are monodispersed, nanoscopic systems, and commonly referred as homogeneous [51]. In 1985, Donald A. Tomalia introduced poly(amidoamine) (PAMAM) dendrimer. Tomalia coined 'starburst polymers' as a new polymers class. This imperative contribution of Tomalia and co-workers opened up a whole new explorative field of nanotechnology [52]. Dendrimer shows a rising class of low polydispersity and hyperbranched macromolecules that presents unique highlights, for example, noteworthy control on molecule size, great branching density, nanoscale size and high surface functional groups [52, 53]. Dendrimers represent more diversity of chemical structure, as poly(etherhydroxylamine) (PEHAM), poly(propylenimine) (PPI), poly(amidoamine) (PAMAM) and poly(L-lysine) (PLL) and polyester dendrimers [54-56]. Dendrimers show impressively improved chemical and physical properties in contrast to the conventional direct polymers. Dendrimers have various noteworthy properties that makes them more interesting such as (i) potential to encapsulate drugs with poor solubility profile within the inner cavities, (ii) nanosize and short polydispersity index (PDI) of dendrimer, take-up by the reticuloendothelial framework can be kept away, (iii) better solvency, retention effect and penetrability, (iv) neutral and anionic surface dendrimers makes them less cytotoxic and low visual irritation, (v) targeting efficacy, (vi) biocompatibility, and (vii) manageable periphery charge [57, 58].

Numerous investigations revealed that dendrimers can be used for many purposes, for example, in delivery of drugs, genes, antioxidants, peptides, and in biomedical imaging. Dendrimers include a nanoscopic class of compounds, with characteristic monodispersed and homogeneous molecular structure. Being referred as fourth and most up to date polymers class [59, 60], these dendrimers vary from the traditional oligomers/polymers because of their symmetry, great branching, and a high end-functional density.

There are several methodologies for dendrimer fabrication, however divergent and convergent techniques are more commonly utilized methods [45, 61]. Over the most recent decade, new methods have been introduced, for example, lego chemistry, joined convergent-divergent, hyper cores, click synthesis method, double exponential and branched monomers method [62, 63]. To date, more than 50 dendrimers families exist, with different and unique properties, since the interior, surface and center can be designed for different types of dendrimer application [64].

4.1.1 Comparative account of linear polymer, dendritic polymer and dendrimers

Linear polymer does not have branched structure and shows better thermostability over the branched (or cross-linked) polymer, which demonstrates that ladder type structure of polymer successfully expands the thermal stability [61].

Dendritic polymers are new class of synthetic polymers derived by structures reminiscent of trees. These are diversified, highly branched structures supporting the cavities formation and thus recent encapsulation properties for different organic and inorganic molecules. Dendritic polymers do not possess the perfect dendrimer branched structure; they can be easier to prepare and still maintain most of the positive dendrimer attributes, such as high surface functionality. Appropriate chemical functionalization gives proper structural features, for example polarity, flexibility, and potential intermolecular interactions with these substances [45]. Dendritic

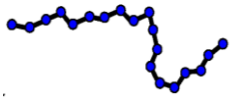
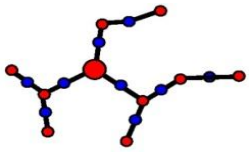
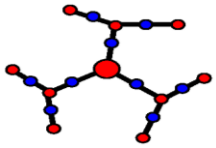
polymers considered as branched polymeric architectures, are classified into random hyperbranched polymers, dendrigraft polymers, dendrons, and dendrimers based on degree of structural control [55]. In general, branched polymers have sophisticated topological structures and exciting physico-chemical and biological properties. In comparison to their linear analogues, branched polymers have three-dimensional globular structure, lower solution/ melting viscosity, smaller hydrodynamic radius, higher degree of functionality, increased encapsulation capabilities, enhanced solubility and minimal molecular entanglement.

Dendrigraft polymers have less controlled structures. The branching densities cannot be determined exactly, they are somewhat arbitrary and difficult to control. Dendrigraft polymers are having more flexible and extended structures. They exhibit unique and different properties compared to the more compact traditional dendrimers. Dendrigraft polymers can be generated through ionic polymerization by combining the features common to dendrimers and random hyperbranched polymers [45].

Dendrimers are artificial polymers with interesting structural properties which have been broadly explored for their biomedical applications, particularly in drug delivery [46]. Monodisperse nature of dendrimers, in addition, provides reproducible pharmacokinetic behavior as compared to linear polymers, which are generally polydisperse and thus containing varying molecular weighed fractions among a given sample. Also, controlled globular shape of dendrimers other than entangled and coiled structures of linear polymers enhances their biological properties [58]. Furthermore, prospect of surface engineering due their high density of functionalities allow to tune their thermal, mechanical, rheological, solution properties (size, conformation, solubility), and biocompatibility. These features can further improve the biodistribution and pharmacokinetic profile, tendency of crossing biological barriers, and blood

circulation time [61, 62]. Different properties of linear polymer, hyper-branched polymer and dendrimer are represented in Table 1.

Table 1: Various properties of linear polymer, hyper-branched polymer and dendrimer

Properties	Linear Polymer	Hyper-branched Polymer	Dendrimer
Structure			
Topology	3D, irregular	3D, regular	1D, linear
Synthesis	One-step, relatively facile	Multiple-step, laborious	One-step, facile
Purification	Precipitation	Chromatography	Precipitation
Scaling-up	Already, easy	Difficult	Already, easy
Molecular weight	Discrepant	Identical	Discrepant
Polydispersity index	>1.1	1.0 (<1.05)	>1.1
Degree of branching	0.4-0.6	1.0	0
Entanglement	Weak	Very weak or no	Strong
Viscosity	Low	Very low	High
Solubility	High	High	Low
Functional group	At linear and terminal units	On periphery (terminal units)	At two ends

Reactivity	High	High	Low
Strength	Low	Very low	High

4.2. Structure of Dendrimers

The dendrimers structures are made up of multifunctional core, which permits the branch coupling, repeated branches layers originated from core known as dendrons and functional groups are present on the surface [65-67]. The first union consisting of a core substituted with dendrons leads to the first generation of the dendrimers. By increasing the number of branches in the structure of dendrimer, a higher generation of dendrimer can be acquired. Hence, the second layer of repeated units prompts the second generation of dendrimer and thus afterwards. With respect to the dendrimer fabrication, these compounds can be synthesized mainly by convergent or divergent methodologies [48]. Generally, surface groups are either neutral, or charged (anionic/cationic) and the toxicity results showed that cationic dendrimers are most cytotoxic among all the dendrimers [68]. To address the challenge of cytotoxicity incited with dendrimers, different approaches have been proposed dependent on the suppression of cationic surface, which can be achieved via chemical adjustment, PEGylation and acetylation with neutral or anionic compounds. It is likewise essential to accentuate the multifunctional dendrimers character, permit the linkage of various ligands of numerous receptors, accomplishing the selectivity along with even synergistic impact [69, 70]. Covalently planned dendritic macromolecules demonstrate the advantage of progressive control over the drug release [71].

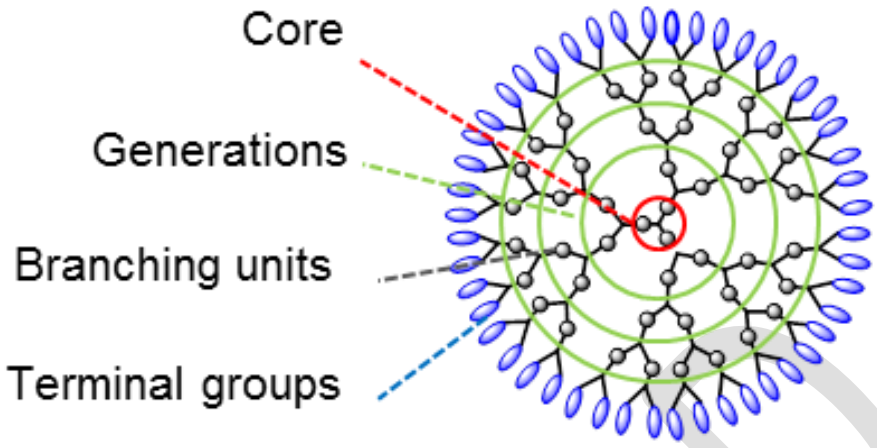


Fig. 2. Typical dendrimer structure

4.3. Types of Dendrimers

4.3.1. Poly (propylene imine) dendrimers

Poly(propylene imine) (PPI) is the earliest known dendrimer presented by Vogtle defining the propylamine spacer moieties. Generally, comprised of poly-alkylamines with primary amines end groups and inside is comprised of some of tertiary tris-propyleneamines. These types of dendrimers have been examined in organic and material sciences. Occasionally, "diamino butane" (DAB) and "polypropylene amine" (POPAM) dendrimer are likewise utilized as another term for PPI dendrimers. Its subdivision is Polyethylene imine (PEI) dendrimers, comprised of diamino propane or diaminoethane as central core's functional group [72].

4.3.2. Poly(amidoamine) dendrimers

Poly(amidoamine) (PAMAM) is a class of dendrimer consisting tertiary amines and polyamide as branching points. Tomalia and colleagues presented PAMAM dendrimers in mid-1980s later than they were considered broadly by scientists. "Starburst" dendrimers, the PAMAM sub-class trademark consist of tris-aminoethylene-imine group as center core. This name was given because of their star-like outer shell of high-generation of dendrimer structure when examined 2D [72].

4.3.3. Frechet-type dendrimers

Frechet-type dendrimers are the type of dendrimer, establish by Hawker and Frechet with hyper-branched engineering of poly-benzyl ether. It includes carboxyl (–COOH) as end groups and consequently offering superior branching point intended for modulation of functionalization of end groups. Likewise, the existence of these polar end groups helps to increase the solubility of this dendrimers in aqueous and polar media [44].

4.3.4. Core shell tecto-dendrimer

Core shell tecto-dendrimers are the dendritic structures where dendrimer molecule is utilized as a center encompassed covalently through different dendrimers shell. As a rule, the center generation number is greater than the encompassing dendrimers. The connection of extra shells is constrained by engineered techniques permitting development of nanoscale area of 1–100 nm [72].

4.3.5. Chiral dendrimers

Chiral dendrimers are fabricated by utilizing intrinsically differentiating branches, chemically related to the chiral center. Chirality is crucial, besides the axis of functional groups. Seebach and coauthors fabricated chiral dendrimers to examine the impact of chiral building blocks on the dendritic structure's chirality. Moreover, to show the likelihood of enantio particular complexation of host with these models [73]. The chirality and optical property of dendrimers involves chiral center diminished with increment in dendrimer size. As of now, numerous researchers are dealing with the advancement of chiral dendrimers [72].

4.3.6. Liquid crystalline dendrimers

The fabrication of liquid crystals has probable industrial applications and has been determined by many analysts. These dendrimers are comprised of mesogenic liquid crystalline monomers. The

liquid crystalline mesophages or phages are developed by molecules are generally disk-like (discotic) or rod-like (calamitic) for example, carbosilane dendrimers have mesogenic functional groups, for example, cholesteryl and cyanobiphenyl. The racemic AB2 rod synthesis like mesogenic monomer depends on conformational isomerism, 13-hydroxy-1-(4-hydroxyphenyl)-2-(4-hydroxy-4″-p-te-henylyl) tridecan, and formation of its initial four dendrimers and monodendrons generations via convergent method is determined by Percec and co-workers [74]. Pedziwiatr-Werbicka and collaborators estimated the capability of amino ended carbosilane dendrimers to convey short chain of siRNA in the form of dendriplex, which was found to be least cytotoxic for blood cells as compared to the parent dendrimers suggesting its activity in the bioactives delivery [75].

4.3.7. Peptide dendrimers

Peptide dendrimers are spiral or wedge-like branched macromolecular framework containing peptidyl branching center and covalently bonded end-functionalized groups. These peptide dendrimers are fabricated by convergent and divergent technique for production. These have been examined for many biochemical and biotechnological application because of their precise composition and simplicity in production. These dendrimers discover their application as surfactants and in biomedical science field as various peptides antigen, protein mimics and transporter for gene and drug delivery. Darbre and Reymond utilized these dendrimers as catalyst of esterase [76].

4.3.8. Multiple antigen peptide dendrimers

Tam has reported the multiple antigen peptide (MAP) dendrimer, constructed by utilizing skeleton of polylysine. The branching points in the dendrimer structure are presented utilizing

alkyl amino unit of monomer lysine side-chain. These dendrimer has been examined broadly in biomedical science involving diagnostic and antibody study [77].

4.3.9. Glycodendrimers

These dendrimers are the monodispersed macromolecule of dendritic framework involving CO_2 moiety. The vast majority of glycodendrimers concludes saccharide buildup groups of terminal and sugar units in the core. CO_2 centered, CO_2-based, and CO_2 coated dendrimers are the three kinds of glycodendrimers classifications [57], that shows a better relationship with lectins joined frameworks than mono-CO_2 attached frameworks. They are utilized in site-explicit delivery of drug aimed to the organs with rich-lectin.

4.3.10. Hybrid dendrimers

Hybrid dendrimers is a mixture of dendritic and straight polymers, present in hybrid block form or grafted copolymer. Dendritic hybrids are probably framed because of their circular shape and extensive quantity of end-functional groups present on dendrimers. Relationship of the little dendritic piece to the different responsive chain ends makes them widely used for many applications such as surface active agents, compatibilizers or adhesives, or hybrid linear dendritic polymers. Dendritic hybrids have a reduced, unbending, consistently formed globular structure, investigated for different perspectives in the drug delivery field [78, 79].

4.3.11. Polyester dendrimers

The advancement of drugs therapeutic index (TI) is a critical field in diseases like malignant growth, incendiary and different infectious ailments like HIV. These dendrimers are confident here because of its biodegradable and biocompatible properties. As a result of less exposure of drug to healthy tissue, shows less toxicity in contrast to other dendrimers which is an ideal property for any compounds to be used in drug delivery. These dendrimers consist of inner void

spaces undifferentiated from parent dendrimers and hence may be utilized as a nanocarrier for small molecular drugs, imaging moieties or metals [80-82]. The reactive surface of these dendrimers can be modified to accomplish the targeted delivery, enhanced biological distribution, controlled and balanced release of the encapsulated drugs.

4.4. Properties of Dendrimers

Dendrimers are based on their specific nature from intricate resources and their examination converge the exploration fields of both polymer science and organic chemistry. With a developing enthusiasm for finding and using the dendrimers for novel applications, particularly in biomedical sciences, a huge tool compartment of characterization methods is essential to satisfy the stringent quality requirements for such applications. The difficulty caused in determining these materials is frequently an outcome of the similar distinguishing characteristics that make them attractive for broad applications: their definite building, monotonous and layered structure, huge number of functionalized groups, and globular structure in solution. The foundation in dendrimer science is the amalgamation of monodisperse dendrimers, depends on total substitution of the functionalized groups. This is particularly essential for most progressively prevalent divergently developed dendrimers, where the quantity of concurrent responses that must be performed increments for every generation and where division of defective structures is troublesome because of the little polarity and size contrast among completely and incompletely responded dendrimers. Convergently developed dendrimers, require lesser number of synchronous responses to be performed amid each generational step, and have a lot bigger contrasts in size between the completely reactive structure and partially reactive molecule, and as an outcome their partition is easier. A mistake in the convergent synthesis results in a totally missing arm of the dendrimer when contrasted with the minor errors

happening in the divergent case. Thus, reliable techniques for characterizing the response progress, the kind and number of terminal groups, just as the purity of the last product are crucial in dendrimer research [83-86].

4.5. Methods of Synthesis of Dendrimers

4.5.1. Divergent synthesis

The divergent synthesis includes the dendrimer development beginning from the center and working up the molecule to the outer surface in a stepwise manner utilizing dual essential activities. In each stage, a novel layer of branching unit is included, which causes increment by one number of generation in the dendrimer structure. In the main activity, monomer is coupled later the monomer end-assemble is deprotected or changed to make another receptive surface and after that coupling of another monomer, and so on. It is raising combination of dendrimers. In spite of being a the very fundamental method, this methodology requires extremely compelling responses to evade mistakes in the dendrimer structure in light of the fact that expanding number of responses must occur simultaneously with expanding generations. Different dendrimer structures can be worked among divergent strategies such as phosphorus-based dendrimers, PPI, and PAMAM dendrimers.

The divergent dendrimer strategy presents two fundamental challenges. First, the quantity of response focuses rises rapidly amid the formation which causes an increment in the response focuses and in molecular weight. This prompts slower response energy and thereby making it hard to make higher generation of dendritic system. This prompts expanding deletions amid the dendrimer growth causing various imperfections in the dendrimers of higher generation. Besides, the partition of the ideal product from reactants or "cancellation products" ends up difficult because of high molecular resemblance among desirable and obtained results. Notwithstanding

these disadvantages the divergent approach has been practical in the formation of various dendrimer structures [87].

4.5.2. Convergent synthesis

The convergent technique begins at the periphery and continues towards the center for the most part coordinated monomers coupling. A small dendron is fabricated in the starting by connecting two groups at periphery to the branching unit. Afterwards, the two dendrons are connected to the branching unit to frame a dendron of subsequent higher generation. This coupling procedure continued causing multiplying the dendron size every time. In the last stage of the convergent method, core is framed in the last step and at least two dendritic portions (dendrons) are consolidated at the center prompting the development of dendrimer. In this methodology, auxiliary imperfections can be absolutely bypassed since, the quantity of responding accomplices does not change on expanding generations. The quantity of receptive locales stays negligible amid the united generation process which prompts quicker response yields and rates. It decreases the production of dendrimers. Notwithstanding, significant limitation of this technique is the low yield in the combination of higher dendrimers generation and henceforth favored for the development of lower age dendrimers. Dendritic structure formed by convergent strategies is poly (aryl ether) system (Frechet-type) [50]. The schematic portrayal of synthesis strategies for dendrimers is presented in **Fig. 3**.

4.5.3. Hyper cores and branched monomer development

The oligomeric moieties are recently amassed and afterward can be connected together to frame dendrimers in moderately some stages with higher yields [57]. The schematic portrayal of hyper centers and monomer branched development is given in **Fig. 3c**.

4.5.4. Double exponential development

It is practically same as quick development strategy for linear polymers. In this technique, the monomers for divergent and convergent development are set up from a solitary beginning material. The subsequent two materials are then responded together to give symmetrically ensured trimer. This is then utilized for repeating the dendrimer development. Because of quick production feature of the double exponential development approach, it may be effectively coupled with divergent or convergent development [88].

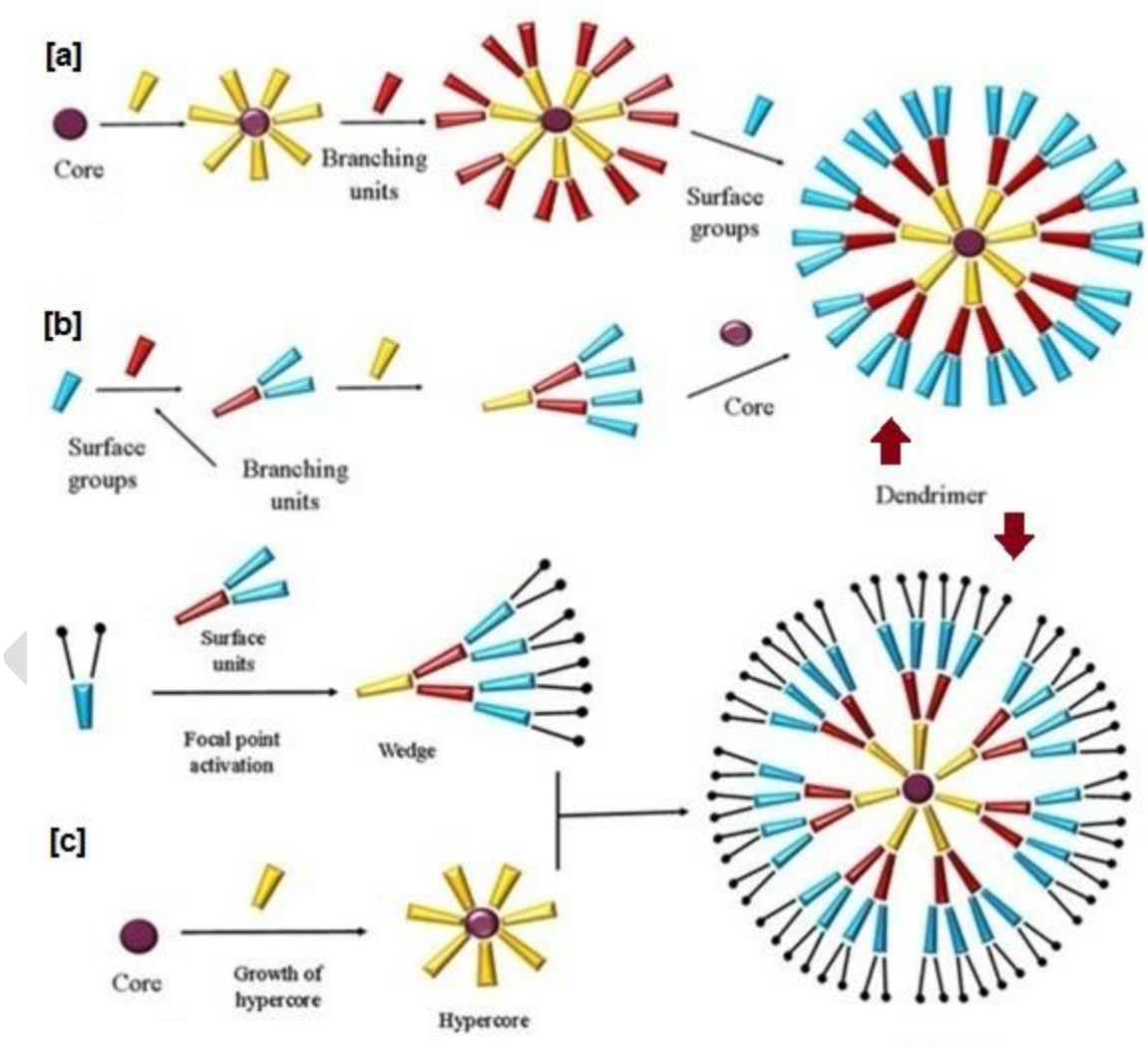


Fig. 3. Common methods of synthesis of dendrimer (a) Divergent (b) Convergent and (c) Hyper cores and branched monomer development [72, 57]

4.5.5. Lego chemistry

In lego science, centers and branched monomers with progressively functionalized groups, are utilized to form phosphorous dendrimers. After couple of varieties when all is said in done engineered planning plan, the set up plan grants multiplications of the quantity of surface dendrimer groups from 48 to 250 out of a solitary advance. Least prerequisite of solvent, simplistic purification probability and development of earth minding side-effects, for example, nitrogen and water are the main highlights of this method [72].

4.5.6. Click chemistry

It is one of the imperative high yielding reactions. Hence, this method has been utilized in amalgamation of numerous new dendritic structures. The strategy is to fabricate carbon-rich dendrimers and natural friendliness. Thiol-yne click responses (TYC), copper-helped azide-alkyne cyclo expansion (CuAAC) [89-91], and thiol-ene click response (TEC) [90, 92] is portion of the generally utilized click responses in the amalgamation of dendrimer. Aside from these responses, new responses are being investigated to additionally enhance the dendritic design [93]. Staudinger response was adjusted to give "click" components. A "Janus" and an "onion peel" dendrimer [94] were formed with this new response. Trans-cyclooctene/tetrazine [95] pair and the Sharpless' new SuFEx response [96] are a portion of alternate responses that shows click qualities and could be utilized for dendrimer fabrication [97, 98]. Mullen et al united folic acid conjugated dendrimer by means of fluoresce in isothiocyanate conjugated dendrimer and showed their receptor based targeting property and fluorescence imaging property. These dendritic units were coupled by utilizing 1,3-dipolar cycloaddition response (click science) amid an azide and alkyne surface groups of first and second dendrimer, respectively. In an *in vitro* study done on

human epithelial malignant growth cell line, it was reported that this coupled dendrimer module can target the over-expression of folic acid receptor [99].

5. DENDRIMERS MEDIATED ANTI-DIABETIC DRUG DELIVERY

5.1. PAMAM dendrimer

Labieniec et al. examined that PAMAM G4 suppresses the plasma hyperglycaemia in diabetic rodents, result in decreased glycoxidation and post-synthetic non-enzymatic alteration of biomacromolecules in sustained streptozotocin-diabetic rodents. In spite of glucose, additional triglycerides and plasma cholesterol, increased in diabetic individuals, reduced upon PAMAM G4 direction [100]. Boas and Heegaard uncovered that the absence of lytic poisonous quality of PAMAM dendrimers up to the fifth era, when surrendered at dosages of 10 mg kg^{-1} to mice [101].

Diabetes-induced damage identified with hyperglycaemia is related to the impaired mitochondrial function. Despite their cytotoxicity, PAMAM G4 dendrimers lowers or suppress sustained markers and plasma glucose of diabetic hyperglycaemia in an experimental diabetic animal model [102].

Nowacka and associates examined differential scanning calorimetry (DSC) sequences of various PAMAM dendrimer generations and predicted that at the lower convergence of G3 and G4 dendrimers (0.06 mol/l), the region under the main peak (at 60°C) diminished while, it expanded under the second peak. Consequences of Thioflavin T (ThT) fluorescence demonstrated that in nearness of G3 and G4 PAMAM dendrimers, at lower concentration the procedure of fibril development is backed off, yet the last measure of fibrils was same as for insulin in the absence of dendrimers. Lower dendrimers concentration can more readily secure the proteins structures.

It is also shown that the individual fibrils packs included 2-5 filaments curved together, their gross distance across being about 12- 20 nm [103].

Akhtar et al. demonstrated that, at low concentrations (1 or 5 mg/kg) PAMAM-induced variations in Mitogen-activated protein kinase (MAPKs) in the diabetic kidneys happened autonomously of EGFR, accordingly suggesting that PAMAMs can prompt uncoupling of MAPK signaling from EGFR at least in this infected state. Additionally, the reality the dendrimer-initiated changes in estimated glomerular filtration rate (eGFR) and p38 MAPK signaling in the segregated ordinary kidney could be turned around by antioxidant treatment recommends that Superfect (SF) and Polyfect (PF) PAMAM dendrimers balanced signaling of MAPK by means of an oxidative-stress dependent manner/pathway [104].

Araujo et al. work reported that not just 99% of PAMAM-CNT-miR-503 framework transfection adequacy, but additionally exceedingly effective micro Ribonucleic acids (miRNA) discharge in endothelial cells, being thusly ready to control the cell multiplication population. The miR-503 stability towards RNAse was likewise significantly increased. At last, the quantity of vessels on a sponge model subcutaneous embed on mice managed with PAMAM-CNT-miR-503 was altogether decreased when contrasted with both the free and the control miR-503 [70].

Dong et al. decided that both the plasma insulin and glucose levels focuses in rodents administered with PAMAM dendrimers and exhibited that the operators adequately expanded the pneumonic retention of insulin with no membrane harm to the respiratory tissues. This astounding hypoglycaemic impact, seen after pneumonic organization of insulin with PAMAM dendrimers, demonstrates that dendrimers may perhaps be utilized as transporters in anti-diabetic treatment [105]. Labieniec-Watala proposed that various metabolic impairments of untreated and durable exploratory Streptozotocin (STZ)-induced DM in rodents can be viably diminished and

controlled by the means of subcutaneous or intraperitoneal infusion of PAMAM dendrimers [106]. Siewiera and Labieniec-Watala research dependent on PAMAM G3 dendrimer effectiveness to enhance the weakened breath of rodent heart mitochondria. They uncovered that dendrimer G3 (20 mg/kg body weight) is poisonous and has a high mortality among the animals controlled with G3 did not permit to play out a dependable result investigation [107].

5.2. Triazine dendrimers

Fluorescence-based glucose observing systems, in light of an aggressive restricting association between concanavalin A (Con An) and CO_2, for example, glucose, dextran, and mannose, are non-obtrusive, and offer an open door for continuous blood glucose monitoring [108]. A triazine dendrimer having 12 groups of glucose arranged and examined for an aggressive restricting measure with a fluorescently-marked Con A. To assess its potential as an implantable sensor, a microporous polyethylene glycol (PEG) based hydrogel circle bearing the dye marked Con An and glycosylated triazine dendrimer was intensely titrated using glucose. The fluorescence power titration plot against glucose focus seemed linear inside the concentration of glucose 50–200 mg/dL with an affectability of 1.3% per 10 mg/dL [109].

5.3. Cationic dendrimers

The advancement of a productive strategy to enhance the injury healing process is critically required for diabetic patients enduring risk of appendage removals. Different development factors have been introduced for the management; in any case, more research still must be done to keep up their healing impact. Kwon et al. built up an advance cationic dendrimer, L-arginine-joined poly-amidoamine (PAM-RG4), for the safe and effective DNA conveyance. Since, the PAM-RG4 interior comprises of an exceedingly requested PAMAM dendrimer base, PAM-RG4 demonstrates a limited conveyance of molecular loads [Mn=22 560; PD (Mw/Mn)=1.063] and

comparing uniform physicochemical characteristics. Furthermore, 62 arginine buildups on the PAM-RG4 surface and give more effectiveness of cell delivery. Consequently, it is found that effective multiplying cells in wound tissue were proficiently transfected, bringing about a high state of vascular endothelial growth factor (VEGF) expression. Six days post injection, diabetic mice skin wounds were commonly mended and shown a well-arranged dermal structure, which was further affirmed by histological stain [110].

6. TOXICOLOGICAL ASPECTS OF DENDRIMERS

Akhtar and collaborators found that intense *in vivo* administration of cationic PAMAM dendrimer (naked) delivery frameworks, at doses that don't indicate net poisonous quality (at the morphology of kidney level), can inherently balance renal signaling of MAPK-an essential cell signal transduction path [104].

Labieniec et al. showed that most of the respiratory parameters of mitochondria were lesser in rodents with diabetes in contrast to the control non-diabetic animals, which implies that the mitochondrial breath limit under diabetic conditions is unequivocally hindered. Regardless of the useful impacts of dendrimers on glycaemic control, these specialists weakened the capacity of heart mitochondria, to a considerably greater degree as of diabetes itself. This implies paying little attention to normalizing the signs of diabetes seriousness, PAMAM G4 did not re-establish the heart mitochondrial breath limit evidently diminished in diabetic rodents, which may point to a lethal impact of the PAMAM dendrimers on heart mitochondria [102].

7. SURFACE ENGINEERING OF DENDRIMERS

Chemically, the drug molecules conjugate with dendrimer surface or encapsulate physically within the core of dendrimer. When functionalized groups are initiated before coupling, a great coupling efficacy might be accomplished. Frequent structural units present in drug polymers and

molecules are carboxyl (COOH), hydroxyl (OH), thiol (SH), guanidine (CH_5N_3) and primary amine (NH_2). The hydroxyl groups can be changed over to dynamic intermediates supporting the nucleophilic responses. Sometimes, the direct coupling might prompt the drugs deactivation, with the goal that the prodrugs won't have any therapeutic impact in anticipation of divided from the holding. The spacer of two functional groups can be acquainted with the targeted delivery or controlled release of therapeutic drugs to form bonds that are enzyme-cleavable or hydrolysable *in vivo* [111].

Dendrimer as core and surface can be tailored using phosphate, amino acids, folate, fluorine, antibody, lipids or RGD (Arg-Gly-Asp). Fluorinated dendrimers shows great small interfering Ribonucleic acid (siRNA) loading capability and electronegativity. Amino acid modified dendrimers are synthesized using endogenous proteins; which improves dendrimer endosomal getaway and biocompatibility. Carbosilane dendrimers raise the ability of tissue's gene transfection to be managed. Phosphate dendrimers have hydrophobic base and the surface is hydrophilic which increases their permeability for the targeted tissue. Lipid based dendrimer undergoes endosomal escape and enhances the dendrimers permeability. Specific tissues targeting achieved via coupling dendrimer by RGD, folate and definite antibody, thus falling off target consequence [112, 113].

The surface engineering of dendrimers is convenient and a graceful approach that covers the dendrimer with positive charge makes it more biomimetic nanocarriers and alters the biological and physicochemical properties of dendrimers. Therefore, dendrimers surface engineering chemistry holds an assurance in the advanced drug encapsulation, solubilization, modified gene transfection, controlled and sustained drug release and intracellular targeting in the investigative field. Multifunctional dendrimer development have promising applications in biomedicines

because targeting ligands quantity determines the narrowness in the similar manner as further kind of group will secure the stability in the biological environment and extended distribution, while others assist their delivery via cell membranes [114].

7.1. Fluorophore-modified dendrimers

PAMAM dendrimers engineered using fluorescence dye, Oregon Green 488, allows the vector visualization during transfection of gene. Unexpectedly, the fluorophore-engineered dendrimer represents greater transfection property as compared to not modified dendrimer, which is similar to 3 commercial transfection compounds and involves Lipofectin, Lipofectamine and SuperFect. The unpredictable elevated fluorophore-modified dendrimer transfection efficacy is accredited to water resistant property of the fluorescent dye [115, 116].

7.2. Aminoglycoside-modified dendrimers

Aminoglycosides, small molecule antibiotics class with for siRNA and DNA biological affinity. These agents are capable to reconcile the macromolecules cellular uptake based on the transporter valency. Aminoglycosides conjugation like neomycin, neamine, and paromomycin, to PAMAM dendrimer appreciably modifies their transfection efficiency. Also, the aminoglycoside-engineered dendrimers can also proceed as antibacterial reagents [115].

The toxic PAMAM dendrimers can gradually turned to be “more friendly” and safer for cellular environment on enduring non enzymatic N-glycosylation in existence of extreme glucose (like in severe hyperglycaemia or diabetes conditions). Labieniec et al., have shown that trial model of streptozotocin-caused diabetes in rodents: higher the hyperglycaemia extents in diabetic animals revealed as more protective for the analyzed toxicity of PAMAM [100].

For dendrimers, N-glycosylated or glycated dendrimers becomes the final product in the non-enzymatic glycosylation (glycation, N-glycosylation) process, characterized as a dynamic energy

for amino acid glycation. In their previous study, Labieniec-Watala and Watala demonstrated that in *in vitro* study that the parent PAMAM dendrimers (full-generation) have the ability to interact with molecules of glucose and make stable bonds. The non-enzymatic reaction amid the amine-end and glucose groups of dendrimers can undergo glycated/N-glycosylated dendrimers formation. Similarly, PAMAM dendrimers can serve as glucose hunter in hyperglycaemia, therefore resulted as reduced protein glycooxidation and glycation. The final N-glycosylated dendrimers of nonenzymatic N-glycosylation, neither result of fabricated and oriental chemical synthesis, their result became the potential applications source in several biomedicine branches [117].

7.3. L-lysine modified PAMAM dendrimers

The slightly modified amino terminal on surface of PAMAM acts as a good alternative in treating metabolic diabetes impairments. The modified, totally theoretical thought, seems believable to modify parent amino-terminal PAMAM to bind with lysine residues, result in "polylysylated" PAMAM dendrimers. These dendrimers attain 2 new liberated amine "valencies" of L-lysine (and g) for each one engaged primary amine group on the surface of the dendrimer, reasonably predictable that L-lysine modified PAMAM dendrimers become more effective in excessive glucose scavenging as compared to their non modified counter parts. When the non-enzymatic glycosylation process takes place accurately at the g amine groups of lysine residues on interactions with glucose, assumed that PAMAM dendrimer loaded with residues of lysine on dendrimer's surfaces may be an effective and ideal focus in diminishing the post enzymatic proteins modifications, and tentatively more suitable for scavenging than non-lysylated dendrimers [117].

7.4. PEG modified PAMAM dendrimer

The cationic dendrimer modification using other molecules decreases the positive charge present on dendrimer's surface, and cause a gradual reduction in cytotoxicity. Therefore, the dendrimer's biological profile depending upon delivery systems differ from dendrimers with modified and unmodified surface [118]. Certainly, this has been established for complexes of DNA/PAMAM dendrimer [119] and oligonucleotide-dendrimer [116]. Between numerous methods yet to be researched, to overcome their significant limitation, the dendrimers modification via the attachment of PEG (polyethylene glycol) on dendrimers' surface appears to be the most prominent [120]. There are unresolved questionnaire and facts of dendrimer systems intended for pharmaceutical applications due to their great water solubility, biological compatibility and capability to advance the biological carriers distribution [121]. Kim et al. demonstrated several examples to attach PEG on dendrimers surface by formation of different bonds, forming hybrids to different geometries, in which the drug delivery application is most prevailing [122]. Due to the polycationic PAMAM dendrimers toxicity, different approaches have been suggested to obscure the end amino groups. Between them, PAMAM's surfaces partial acetylation, in which the toxic fraction of amino groups was left unrevealed to accomplish attractive properties, deduct the hydrodynamic degree and affects the water solubility [123, 124].

7.5. Lauroyl modified cationic PAMAM dendrimers

Partial alteration of cationic PAMAM dendrimers to lauroyl terminal groups improved the permeability of membrane and reduced the cytotoxicity [118, 125]. The modification also enhances hydrophobicity, and support water aggregation by conjugated aliphatic chains. For example, modification using small alkyl alcohol groups points to reduce the cytotoxicity and also maintained the water solubility [124, 126]. Moreover, modification of PAMAM surface through moderately small functional groups needs complex regulation of stoichiometry for every

appended functional moiety to get both the pharmacological and physicochemical properties. A noticeable fall in the cationic PAMAM dendrimers cytotoxicity seemed, cells came in contact of lauroyl-PAMAM dendrimers. While concluded from estimated IC_{50} values, the cytotoxicity level of PAMAM G3 and G4 dendrimers conjugates by six lipid chains attached was >7-fold decreased as compared to unmodified dendrimers [127].

7.6. Acetylated PAMAM dendrimers

Acetylation of the reactive terminal groups of PAMAM dendrimers, route to particularly functionalize primary surface amines. When PAMAM dendrimers are acetylated, they become more water-soluble and the resulted quality is important for the biomedical applications which require more solubility in aqueous solvents [124, 127]. Though, systematic study of the science is not involved in PAMAMs acetylation that has been done. Majoros et al., conducted the primary study associated with PAMAM G5 acetylation, designed their protocol to research on the acetylation reaction's nature has been used, as well as to yield an investigation of well-defined conjugates for dendrimer to use in the biomedical science field. Depending upon the data obtained by several techniques, like potentiometric titration, ^{1}H NMR, ^{13}C NMR and Gel permeation chromatography (GPC), the researchers concluded that the acetylated dendrimers unconditionally exhibited small molecular size in spite of their high molecular weight. Furthermore, they represented a highly compact design as compared to their non-acetylated analogues. However, the conversion of all primary amines into acetyl derivatives changes the dendrimer structure. The re-location of NMR characterized spectral peaks and appearance of some new peaks, concludes that modification of PAMAM G5 considered as a controlled and stoichiometric reaction (ratio of primary amine and acetic anhydride is 1:1). This information showed that stoichiometric property of the acetylation reaction, with an understanding of the

accurate amount of primary amine surface groups, confirmed a defined chemical structure for other dendrimer conjugates [126]. Kolhathar et al. studied two generations of PAMAM dendrimers (G2 and G4) and acetylated dendrimers and the result of surface advancement on permeability, uptake and cytotoxicity was determined through Caco-2 epithelial cells [128, 129]. These authors also showed that cationic PAMAM dendrimers can permeate across the Caco-2 cells to a greater extent as their anionic or neutral counterparts [129]. The factors prompted the use of acetyl group for the surface functionalization are (1) an attractive interest in surface modification of dendrimers used for drug delivery, (2) simplicity of controlling the acetylation degree by regulating stoichiometry, (3) mild reaction environments requisite for acetylation, and (4) improved acetylated dendrimers solubility.

The acetylated dendrimers shows less cytotoxicity and maintains good permeability. It is possible to make surface-modified PAMAM dendrimers specified for biomedical applications [128, 129]. Quintana et al used acetylation to reduce the non-definite binding linked with amino terminated nanoparticles [124]. Wiwattanapattapee et al. observed greater tissue uptake and reduced rate of serosal transfer for surface modified dendrimers as comparison to the non-modified dendrimers [130]. PAMAM dendrimers consist of large number of tertiary amino groups inside, interacting with negative charged cell membranes and enhance the permeability. In animals PAMAM G4 (7-8 mg kg-1, c.a. 5.8 μM in circulating blood) was administered daily and revealed enhanced glycaemic control diabetes markers, though PAMAM-managed rats showed improved metabolic control of long term hyperglycaemia. Higher blood glucose level was established as protective counter to polycationic PAMAM-mediated increased mortality and cytotoxicity under chronic diabetes conditions [99].

7.7. Arginine modified PAMAM dendrimer

In order to build up the transfection efficiency aimed for PAMAM dendrimers, different ligands were attached. A great deal of study has been done to introduce arginine in polymers like PAMAM dendrimers to enhance their transfection efficiency [131, 132]. Nam et al. confirmed that PAMAM dendrimers (G3 and G4) functionalized with lysine (PAMAM-K) or arginine (PAMAM-R) via an amide bound showed a better transfection applications as compared to the plain G3 and G4 [133, 134]. Furthermore, researchers noticed that PAMAM dendrimers can be modified using arginine by biodegradable ester bond for two purposes. First, the attached arginine affects the formation of polyplex and penetrates the nucleus and cell membrane. Second, the polymers biodegradability affects the efficacy of intracellular disassembly for siRNAs and oligonucleotides as well as cell viability [135].

7.8. Arginine modified PAMAM-OH dendrimer

PAMAM-OH dendrimers having similar structure as PAMAM-NH_2, excluding the surface-NH_2 groups of cationic dendrimers are exchanged for -OH groups. PAMAM-OH is non-toxic because of the lack of primary surface amines; however it barely forms DNA polyplexes due to a low pKa value of the internal tertiary amines. To manage this, biodegradable arginine (A)- and lysine (K)- conjugated constructed PAMAM dendrimers with ester bonds amid the surface -OH groups of PAMAM-OH and carboxylate groups of arginine and lysine (alluded as e-PAM-A and e-PAM-K, respectively). Hence, it is concluded that PAMAM-OH dendrimers (especially arginine surface modified) have tremendous potential to become a safe and effective carrier of gene delivery with a high transfection efficiency, biodegradability, and low cytotoxicity [133]. Pisal et al., studied the permeability across the Caco-2 cell monolayers, and the cytotoxicity of PAMAM G4 dendrimers attached with ornithine and arginine. Their results showed that surface modified dendrimers were transported across the epithelial monolayers notably faster as compared to their

unmodified counterparts [135]. The result showed that PAMAM dendrimers simply modified at their surfaces by glycation, acetylation or acylation process; and these modifications make PAMAM dendrimers the least cytotoxic and more valuable for specific biomedical applications, e.g. gene or oral drug delivery [136]. When cationic polymers formed complexes, that shows less cytotoxicity in contrast to cationic free polymers [134].

8. CONCLUSION

Dendrimers provides a strong platform for the delivery of drug molecules and bioactives. Dendrimers are monodisperse macromolecules with the presence of large number of surface groups which increases with the number of generation. The surface modification of dendrimers provides an opportunity for designing the carrier system with desired properties. Dendrimers can work as a useful tool for optimizing the drug delivery system. With the help of surface groups (including high density), a tunable toxicity profile of dendrimers as well as the targeting is possible. So, it is expected that in coming years, the researchers will achieve success to develop dendrimer based novel antidiabetic therapeutics which could be effective to control blood glucose level as well as complications associated with diabetes.

Despite the broad endeavors and the advancement accomplished so far, the issues of a reliable technique for assessing nontoxic dosages of the tested PAMAM dendrimers are still undeveloped. Therefore, the main aim for future research ought to be the estimation of ideal dosages, at which dendrimers can be applied with the required effect. The quantity of laboratories directing *in-vivo* examinations with dendrimers is expanding gradually; the most appropriate (effective and non-toxic) method for administrations of dendrimers are still in need to be developed. Because of the ongoing endeavors for assessing the best technique for dendrimer DDS to organism, we consider the improvement and evaluating new methods of

dendrimer administration to be absolutely critical, featuring that the dendrimer route of delivery to targeted sites shows great impacts on the nature of treatment.

A large portion of the results with respect to the activity and properties of dendrimers is taken from the results of *in-vitro* examinations. But, the results observed from the *in-vitro* and *in-vivo* examinations correspond poorly; subsequently, further examinations ought to be done so as to define and additionally exploit this tendency. Though, various reports appeared to be empowering and raise confidence for the potential utilization of dendrimers in an assortment of medicinal applications, their surprising expense of the fabrication and, subsequently, their high cost, may truly restrict the dendrimers use in the treatment of diabetes.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

1. Kliegman RM, Stanton BM, Geme JS, Schor NF. Nelson Textbook of Pediatrics E-Book: 2-Volume Set. Elsevier Health Sciences 2016; 2760-90.
2. Veiseh O, Tang BC, Whitehead KA, Anderson DG, Langer R. Managing diabetes with nanomedicine: challenges and opportunities. Nat Rev Drug Discov 2015; 14(1): 45-57
3. Hu C, Jia W. Therapeutic medications against diabetes: What we have and what we expect. Adv Drug Deliv Rev 2018. doi: 10.1016/j.addr.2018.11.008
4. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Medicine 2010; 38: 602-06.
5. Flaws B, Kuchinski LM, Casanas R. The Treatment of diabetes mellitus with Chinese Medicine: A textbook & clinical manual. Blue Poppy Enterprises, Inc 2002; 225-32.

6. Tan SY, Wong JLM, Sim YJ, *et al.* Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. Diabetes Metab Syndr: Clin Res Rev 2019; 13: 364-72.
7. Lin C, Gokhale R, Trivedi JS, Ranade V. Recent strategies and methods for improving insulin delivery. Drug Dev Res 2004; 63: 151–60.
8. Groenning M, Frokjaer S, Vestergaard B. Formation mechanism of insulin fibrils and structural aspects of the insulin fibrillation process. Curr Protein Pept Sci 2009; 10: 509-28.
9. M Fändrich. On the structural definition of amyloid fibrils and other polypeptide aggregates. Cell Mol Life Sci 2007; 64: 2066–78.
10. Rekas V, Lo GE, Gadd R, Cappai SI. PAMAM dendrimers as potential agents against fibrillation of Synuclein, a Parkinson's disease-related protein. Macromol Biosci 2009; 9: 230-38.
11. Onkamo P, Vaananen S, Karvonen M Tuomilehto J. Worldwide increase in incidence of Type I diabetes--the analysis of the data on published incidence trends. Diabetologia 1999; 42: 1395-1403.
12. Kesharwani P, Gorain B, Low SY, *et al.* Nanotechnology based approaches for anti-diabetic drugs delivery. Diabetes Res Clin Pract 2018; 136: 52-77.
13. Khalil H. Diabetes microvascular complications-a clinical update. Diabetes Metab Syndr Clin Res Rev 2017; 11: S133-9.
14. Papatheodorou K, Papanas N, Banach M, Papazoglou D, Edmonds M. Complications of Diabetes. J Diabetes Res 2016; 1-3.
15. Ahmad Z, Rasouli M, Azman AZF, Omar AR. Evaluation of insulin expression and secretion in genetically engineered gut K and L-cells. BMC Biotechnol 2012; 12: 1-9.

16. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complicationsin diabetes mellitus: Distinct or continum? Indian J Endocrinol Metab 2016; 20: 546-51.
17. Sharma RB, Alonso LC. Lipotoxicity in the pancreatic beta cell: not just survival and function, but proliferation as well? Curr Diab Rep 2014; 14: 492.
18. Roep BO, Peakman M. Antigen targets of type 1 diabetes autoimmunity. Cold Spring Harb Perspect Med 2012; 2: 1-14.
19. Yang LJ. Big mac attack: does it play a direct role for monocytes/macrophages in type diabetes?. Diabetes 2008; 57: 2922-3.
20. Moullé VS, Vivot K, Tremblay C, Zarrouki B, Ghislain J, Poitout V. Glucose and fatty acids synergistically and reversibly promote beta cell proliferation in rats. Diabetologia 2017; 60: 879-88.
21. Cernea S, Dobreanu M. Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. Biochem Medica 2013; 23: 266-80.
22. Collier JJ, Sparer TE, Karlstad MD, Burke SJ. Pancreatic islet inflammation: an emerging role for chemokines. J Mol Endocrinol 2017; 59: R33-46.
23. Artham SM, Lavie CJ, Milani RV, Ventura HO. Obesity and hypertension, heart failure, and coronary heart disease—risk factor, paradox, and recommendations for weight loss. Ochsner J 2009; 9(3): 124-32.
24. Wang X, Bao W, Liu J, *et al*. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes care 2013; 36(1):166-75..
25. Zhang Y, Wang P, Heaton A, Winkler H. Health information searching behavior in MedlinePlus and the impact of tasks. In Proceedings of the 2nd ACM SIGHIT International Health Informatics Symposium 2012; 641-50.

26. Amery C, Nattras M. Fatty acids and insulin secretion. Diabetes Obes Metab 2000; 2: 213-31.

27. Montane J. Stress and the inflammatory process: a major cause of pancreatic cell death in type 2 diabetes. Diabetes Metab Syndr Obes 2014; 7: 25–34.

28. Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. Exp Mol Med 2016; 48: e219.

29. Holst JJ. Incretin hormones and the satiation signal. Int J Obes 2013; 37: 1161–8.

30. Zand H, Morshedzadeh N, Naghashian F. Signaling pathways linking inflammation to insulin resistance. Diabetes Metab Syndr Clin Res Rev 2017; 11: S307–9.

31. Xu X, Ren J. Macrophage migration inhibitory factor (MIF) knockout preserves cardiac homeostasis through alleviating Akt-mediated myocardial autophagy suppression in high-fat diet-induced obesity. Int J Obes 2015; 39: 387–96.

32. Li Y, Soos TJ, Li X, *et al.* Protein kinase C inhibits insulin signaling by phosphorylating IRS1 at Ser1101. J Biol Chem 2004; 279: 45304–7.

33. Beers MH, Berkow R. (Eds.) The Merck manual of diagnosis and therapy. Whitehouse Station, NJ: Merck and Co. Inc 1999.

34. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 2013; 4: 46-57.

35. International Diabetes Federation. Types of diabetes, http://www.idf.org/types-diabetes. (Accessed on 3 February 2019).

36. Mas A, Montane J, Anguela XM, *et al.* Reversal of type 1 diabetes by engineering a glucose sensor in skeletal muscle. Diabetes 2006; 55: 1546–53.

37. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014; 37: S81-S90.
38. Taplin CE, Barker JM. Autoantibodies in type 1 diabetes. J Autoimmun. 2008; 41(1): 11-8.
39. Dharmananda S. Treatment of diabetes with Chinese herbs and acupuncture. Institute for Traditional Medicine (ITM), Portland, Oregon 2002.
40. Derosa G. Efficacy and tolerability of pioglitazone in patients with type 2 diabetes mellitus: Comparison with other oral antihyperglycaemic agents. Drugs 2010; 70: 1945–61.
41. Tahrani AA, Piya MK, Barnett AH. Drug evaluation: Vildagliptin metformin single-tablet combination. Adv Ther 2009; 26: 138–54.
42. Lefkovits YR, Stewart ZA, Murphy HR. Gestational diabetes. Medicine 2019; 47(2): 114-8.
43. Egan AM, Dinneen SF. What is diabetes? Medicine 2019; 47: 1-4.
44. Elham A, Sedigheh FA, Abolfazl A, *et al*. Dendrimers: synthesis, applications and properties. Nanoscale Res Lett 2014; 9: 247.
45. Saluja V, Mankoo A, Saraogi GK, *et al*. Smart dendrimers: Synergizing the targeting of anticancer bioactives. J Drug Deliv Sci Technol 2019; 52: 15-26.
46. Huang D, Wu D. Biodegradable dendrimers for drug delivery. Mater Sci Eng C 2018; 90: 713-27.
47. Vogtle F, Buhleier EW, Wehner W. Cascade and nonskid-chain-like syntheses of molecular cavity topologies. Synthesis 1978; 2: 155–8.
48. Tomalia DA, Baker H, Dewald J, *et al*. A new class of polymers: starburst-dendritic macromolecules. Polym J 1985; 17: 117–32.

49. De Brabander-van den Berg EMM, Meijer EW. Poly(propylene imine) dendrimers: large-scale synthesis by hetereogeneously catalyzed hydrogenations. Angew Chemi Int Ed Engl. 1993; 32: 1308-11.
50. Newkome GR, Yao Z, Baker GR, Gupta VK. Micelles. Part 1. Cascade molecules: A new approach to micelles. A [27]-arborol. J Org Chem 1985; 50: 2003-4.
51. Mishra V, Gupta U, Jain NK. Influence of different generations of poly (propylene imine) dendrimers on human erythrocytes. Pharmazie 2010; 65(12): 891-5.
52. Tomalia DA. Birth of a new macromolecular architecture: Dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. Prog Polym Sci 2005; 30: 294-324.
53. Abbasi E, Aval SF, Akbarzadeh A, *et al.* Dendrimers: Synthesis, applications, and properties. Nanoscale Res Lett 2014; 9: 247.
54. Taghavi N, Azar P, Mutlu P, Khodadust R, Gunduz U. Poly(amidoamine) (PAMAM) nanoparticles: Synthesis and biomedical applications. Hacettepe J Biol Chem 2013; 41: 289-99.
55. Tomalia DA, Fréchet JMJ. Discovery of dendrimers and dendritic polymers: A brief historical perspective. J Polym Sci Part A Polym Chem 2002; 40: 2719-28.
56. Kesharwani P, Jain K, Jain NK. Dendrimer as nanocarrier for drug delivery. Prog Polym Sci 2014; 39: 268-307.
57. Nanjwade BK, Bechra HM, Derkara GK, Manvi FV, Nanjwade VK. Dendrimers: emerging polymers for drug-delivery systems. Eur J Pharm Sci 2009; 38: 185-96.
58. Gillies ER, Fréchet JMJ. Dendrimers and dendritic polymers in drug delivery., Drug Discov Today 2005; 10: 35-43.

59. Cheng Y, Xu Z, Ma M, Xu T. Dendrimers as drug carriers: Applications in different routes of drug administration. J Pharm Sci 2007; 97: 123-43.
60. Svenson S. Dendrimers as versatile platform in drug delivery applications. Eur J Pharm Biopharm 2009; 71: 445-62.
61. Duro-Castano A, Movellan J, Vicent MJ. Smart branched polymer drug conjugates as nano-sized drug delivery systems, Biomater. Sci. 2015; 3: 1321-34.
62. Myung JH, Hsu HJ, Bugno J, Tam KA, Hong S. Chemical structure and surface modification of dendritic nanomaterials tailored for therapeutic and diagnostic applications. Curr Top Med Chem 2017;17: 1542-54.
63. Kannan RM, Nance E, Kannan S, Tomalia DA. Emerging concepts in dendrimer-based nanomedicine: From design principles to clinical applications. J Intern Med 2014; 276: 579-617.
64. Fischer M, Vogtle F. Dendrimers: from design to application- a progress report. Angew Chem Int Ed 1999; 38: 884-905.
65. Maiti PK, Çagin T, Wang G, Goddard WA. Structure of PAMAM dendrimers: Generations 1 through 11. Macromol 2004; 37: 6236-54.
66. Santos SS, Ferreira EI, Giarolla J. Dendrimer prodrugs. Molecules 2016; 21: 686.
67. Menjoge AR, Kannan RM, Tomalia DA. Dendrimer-based drug and imaging conjugates: Design considerations for nano medical applications. Drug Discov Today 2010; 15: 171-85.
68. Kaminskas LM, Mcleod VM, Porter CJH, Boyd BJ. Association of chemotherapeutic drugs with dendrimer nanocarriers: An assessment of the merits of covalent conjugation compared to non covalent encapsulation. Mol Pharm 2012; 9: 355-73.

69. Hong S, Leroueil PR, Majoros IJ, Orr BG, Baker JR, Holl MMB. The binding avidity of ananoparticle-based multivalent targeted drug delivery platform. Chem Biol 2007; 114: 107-15.

70. Araújo R, Santos S, Igne Ferreira E, Giarolla J. New advances in general biomedical applications of PAMAM dendrimers. Molecules 2018; 23: 2849.

71. Singh AK, Yadav TP, Pandey B, Gupta V, Singh SP. Engineering nanomaterials for smart drug release: Recent advances and challenges. In Applications of targeted nano drugs and delivery systems, Elsevier 2019; 411-49. 10.1016/B978-0-12-814029-1.00015-6

72. Sherje AP, Jadhav M, Dravyakar BR, Kadam D. Dendrimers: a versatile nanocarrier for drug delivery and targeting. Int J Phar 2018; 548: 707-20.

73. Seebach D, Rheiner PB, Greiveldinger G, Butz T, Sellner H. Chiral dendrimers. In Dendrimers. Springer, Berlin, Heidelberg 1998; 125-64.

74. Percec V, Chu P, Ungar G, Zhod J. Rational design of the first nonspherical dendrimer which displays calamitic nematic and smectic thermotropic liquid crystalline phases. J Am Chem Soc 1995; 117: 11441–54.

75. Pedziwiatr-Werbicka E, Fuentes E, Dzmitruk V, *et al.* Novel 'SiC' carbosilane dendrimers as carriers for anti-HIV nucleic acids: studies on complexation and interaction with blood cells. Colloids Surf B 2013; 109C: 183–9.

76. Darbre T, Reymond JL. Peptide dendrimers as artificial enzymes, receptors, and drug delivery agents. Acc Chem Res 2006; 39: 925–34.

77. Tam JP. In: Goodman M. (Ed.). Peptide Dendrimers and Protein Mimetics. Thieme, Stuttgart 2000.

78. Kesharwani P, Jain K, Jain NK. Dendrimer as nanocarrier for drug delivery. Prog Polym Sci 2014; 39: 268-307.
79. Pushechnikov A, Jalisatgi AA, Hawthorne MF. Dendritic closomers: novel spherical hybrid dendrimers. Chem Commun 2013; 49: 3579-81.
80. Twibanire K, Jean-d'Amour G, Bruce T. Polyester dendrimers: smart carriers for drug delivery. Polymers 2014; 6: 179–213.
81. Jain K, Kesharwani P, Gupta U, Jain NK. Dendrimer toxicity: let's meet the challenge. Int J Pharm 2010; 394: 122-42.
82. Antoni P, Hed Y, Nordberg A, *et al*. Bifunctional dendrimers: From robust synthesis and accelerated one-pot post-functionalization strategy to potential applications. Angew Chem Int Ed 2009; 48: 2126-30.
83. Mishra V, Kesharwani P. Dendrimer technologies for brain tumor. Drug Discov Today 2016; 21(5): 766-78.
84. Mishra V, Patil A, Thakur S, Kesharwani P. Carbon dots: emerging theranostic nanoarchitectures. Drug Discov Today 2018; 23(6): 1219-32.
85. Caminade AM, Majoral JP. Engineering CNDP's of dendrimers containing phosphorous interior compositions to produce new emerging properties. J Nanopart Res 2018; 20: 74.
86. Kumar PMK, Kumar P, Choudhary C, *et al*. Dendrimer: a novel polymer for drug delivery. J Innovative Trends Pharm Sci 2010; 1: 252–69.
87. Boas U, Christensen JB, Heegaard PMH. Dendrimers: design, synthesis and chemical properties. Dendrimers in Medicine and Biotechnology. New Molecular Tools. RSC Publishing 2006.

88. Juris A. Recent developments in photo- and redox-active dendrimers. Annu Rep Sect "C" (Phys Chem) 2003; 99: 177-241.
89. Hoogenboom R. Thiol-Yne Chemistry: a powerful tool for creating highly functional materials. Angew Chem Int Ed 2010; 49: 3415-17.
90. Lowe AB. Thiol-ene "click" reactions and recent applications in polymer and materials synthesis. Polym Chem 2010; 1: 17-36.
91. Cervera-Procas R, Sanchez-Somolinos C, Serrano JL, Omenat A. A polymer network prepared by the thiol-yne photocrosslinking of a liquid crystalline dendrimer. Macromol Rapid Commun 2013; 34: 498-503.
92. Lowe AB, Harvison MA. Thiol-based "click" chemistries in polymer: synthesis and modification. Aust J Chem 2010; 63: 1251-66.
93. Svenson S, Tomalia DA. Dendrimers in biomedical applications–reflections on the field. Adv Drug Delivery Rev 2012; 64: 102-15.
94. Gottis S, Rodriguez LI, Laurent R, *et al*. Janus carbosilane/phosphorhydrazone dendrimers synthesized by the "click" Staudinger reaction. Tetrahedron Lett 2013; 54: 6864-7.
95. Katir N, El Brahmi N, El Kadib A, *et al.* Synthesis of onion-peel nanodendritic structures with sequential functional phosphorus diversity. Chem Eur J 2015; 21: 6400-08.
96. Karver MR, Weissleder R, Hilderbrand SA. Bioorthogonal reaction pairs enable simultaneous, selective, multi-target imaging. Angew Chem Int Ed 2012; 51: 920-22.
97. Dong J, Krasnova L, Finn MG, Sharpless KB. Sulfur (VI) fluoride exchange (SuFEx): another good reaction for click chemistry. Angew Chem Int Ed 2014; 53: 9430-48.
98. Becer CR, Hoogenboom R, Schubert US. Click chemistry beyond metal-catalyzed cycloaddition. Angew Chem Int Ed 2009; 48: 4900-08.

99. Arseneault M, Wafer C, Morin JF. Recent advances in click chemistry applied to dendrimer synthesis. Molecules 2015; 20: 9263-9294.

100. Labieniec M, Ulicna O, Vancova O, *et al.* PAMAM G4 dendrimers lower high glucose but do not improve reduced survival in diabetic rats. Int J Pharm 2008; 364: 142-49.

101. Boas U, Heegaard PM. Dendrimers in drug research. Chem Soc Rev 2004; 33: 43-63.

102. Labieniec M, Ulicna O, Vancova O, Kucharska J, Gabryelak T, Watala C. Effect of poly (amido) amine (PAMAM) G4 dendrimer on heart and liver mitochondria in an animal model of diabetes. Cell Biol Int 2010; 34: 89-97.

103. Nowacka O, Milowska K, Belica-Pacha S, *et al.* Generation-dependent effect of PAMAM dendrimers on human insulin fibrillation and thermal stability. Int J Biol Macromol 2016; 82: 54-60.

104. Akhtar S, Al-Zaid B, El-Hashim AZ, Chandrasekhar B, Attur S, Benter IF. Impact of PAMAM delivery systems on signal transduction pathways in vivo: Modulation of ERK1/2 and p38 MAP kinase signaling in the normal and diabetic kidney. Int J Pharm 2016; 514: 353-63.

105. Dong Z, Hamid KA, Gao Y, *et al.* Polyamidoamine dendrimers can improve the pulmonary absorption of insulin and calcitonin in rats. J Pharm Sci 2011; 100: 1866–78.

106. Labieniec-Watala M, Przygodzki T, Sebekova K, Watala C. Can metabolic impairments in experimental diabetes be cured with poly (amido) amine (PAMAM) G4 dendrimers?–In the search for minimizing of the adverse effects of PAMAM administration. Int J Pharm 2014; 464: 152-67.

107. Siewiera K, Labieniec-Watala M. Ambiguous effect of dendrimer PAMAM G3 on rat heart respiration in a model of an experimental diabetes-Objective causes of laboratory misfortune or unpredictable G3 activity? Int J Pharm 2012; 430: 258-65.

108. Moschou EA, Sharma BV, Deo SK, Daunert S. Fluorescence glucose detection: advances toward the ideal in vivo biosensor. J Fluoresc 2004; 14: 535–47.

109. Lim J, Simanek EE. Triazine dendrimers as drug delivery systems: From synthesis to therapy. Adv Drug Deliv Rev 2012; 64: 826-35.

110. Kwon MJ, An S, Choi S, *et al.* Effective healing of diabetic skin wounds by using nonviral gene therapy based on minicircle vascular endothelial growth factor DNA and a cationic dendrimer. J Gene Med 2012; 14: 272-78.

111. Mishra V, Gupta U, Jain NK. Surface-engineered dendrimers: a solution for toxicity issues. J Biomat Sci Polymer Edn 2009; 20: 141-66.

112. Tambe V, Thakkar S, Raval N, Sharma D, Kalia K, Tekade RK. Surface engineered dendrimers in siRNA delivery and gene silencing. Cur Pharm Des 2017; 23: 2952-75.

113. Kesharwani P, Mishra V, Jain NK. Generation dependent hemolytic profile of folate engineered poly(propyleneimine) dendrimer. J Drug Deliv Sci Technol 2015; 28: 1-6.

114. Biswas S, Deshpande PP, Navarro G, Dodwadkar NS, Torchilin VP. Lipid modified triblock PAMAM-based nanocarriers for siRNA drug co-delivery. Biomaterials 2013; 34: 1289-1301.

115. Yang J, Zhang Q, Chang H, Cheng Y. Surface-engineered dendrimers in gene delivery. Chem Rev 2015; 115: 5274-300.

116. Yoo H, Juliano RL. Enhanced delivery of antisense oligonucleotides with fluorophore-conjugated PAMAM dendrimers. Nucleic Acids Res 2000; 28: 4225-31.

117. Labieniec-Watala M, Watala C. PAMAM dendrimers: destined for success or doomed to fail? Plain and modified PAMAM dendrimers in the context of biomedical applications. J Pharm Sci 2015; 104: 2-14.

118. Jevprasesphant R, Penny J, Jalal R, Attwood D, McKeown NB, D'Emanuele A. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. Int J Pharm 2003; 252: 263-66.

119. Brazeau GA, Attia S, Poxon S, Hughes JA. In vitro myotoxicity of selected cationic macromolecules used in non-viral gene delivery. Pharm Res 1998; 15: 680-84.

120. Gupta U, Agashe HB, Asthana A, Jain NK. A review of in vitro-in vivo investigations on dendrimers: the novel nanoscopic drug carriers. Nanomedicine 2006; 2: 66-73.

121. Greenwald RB, Choe YH, McGuire J, Conover CD. Effective drug delivery by PEGylated drug conjugates. Adv Drug Deliv Rev 2003; 55: 217- 50.

122. Kim Y, Klutz AM, Jacobson KA. Systematic investigation of polyamidoamine dendrimers surface-modified with poly(ethylene glycol) for drug delivery applications: synthesis, characterization, and evaluation of cytotoxicity. Bioconjug Chem 2008; 19: 1660-72.

123. Labieniec M, Watala C, Lee H, Larson RG. Molecular dynamics simulations of PAMAM dendrimer-induced pore formation in DPPC bilayers with a coarse-grained model. J Phys Chem B 2006; 110: 18204-11.

124. Quintana A, Raczka E, Piehler L, *et al.* Design and function of a dendrimer based therapeutic nanodevice targeted to tumor cells through the folate receptor. Pharm Res 2002; 19: 1310-16.

125. Jevprasesphant R, Penny J, Attwood D, McKeown NB, D'Emanuele A. Engineering of dendrimer surfaces to enhance transepithelial transport and reduce cytotoxicity. Pharm Res 2003; 20: 1543-50.

126. Majoros IJ, Myc A, Thomas T, Mehta CB, Baker JR. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. Biomacromolecules 2006; 7: 572-79.

127. Thomas TP, Majoros IJ, Kotlyar A, *et al.* Targeting and inhibition of cell growth by an engineered dendritic nanodevice. J Med Chem 2005; 48: 3729-35.

128. Kolhatkar RB, Kitchens KM, Swaan PW, Ghandehari H. Surface acetylation of polyamidoamine (PAMAM) dendrimers decreases cytotoxicity while maintaining membrane permeability. Bioconjug Chem 2007; 18: 2054-60

129. Kitchens KM, Kolhatkar RB, Swaan PW, Eddington ND, Ghandehari H. Transport of poly(amidoamine) dendrimers across Caco-2 cell monolayers: Influence of size, charge and fluorescent labeling. Pharm Res 2006; 23: 2818-26.

130. Wiwattanapatapee R, Lomlim L, Saramunee K. Dendrimers conjugates for colonic delivery of 5-aminosalicylic acid. J Control Release 2003; 88: 1-9.

131. Choi JS, Ko KS, Park JS, Kim YH, Kim SW, Lee M. Dexamethasone conjugated poly(amidoamine) dendrimer as a gene carrier for efficient nuclear translocation. Int J Pharm 2006; 320: 171-8.

132. Gao Y, Xu Z, Chen S, Gu W, Chen L, Li Y. Argininechitosan/DNA self-assemble nanoparticles for gene delivery: In vitro characteristics and transfection efficiency. Int J Pharm 2008; 359: 241-46.

133. Nam HJ, Hahn HJ, Nam K, *et al.* Evaluation of generations 2, 3 and 4 arginine modified PAMAM dendrimers for gene delivery. Int J Pharm 2008; 363: 199-205.

134. Nam HY, Nam K, Hahn HJ, *et al.* Biodegradable PAMAM ester for enhanced transfection efficiency with low cytotoxicity. Biomaterials 2009; 30: 665-73.

135. Pisal DS, Yellepeddi VK, Kumar A, *et al.* Permeability of surface-modified polyamidoamine (PAMAM) dendrimers across Caco-2 cell monolayers. Int J Pharm 2008; 350: 113-21.

136. Dufes C, Uchegbu IF, Schatzlein AG. Dendrimers in gene delivery. Adv Drug Deliv Rev 2005; 57: 2177-202.