Poly(amidoamine) (PAMAM) Nanoparticles: Synthesis and Biomedical Applications

Poli(amidoamin) (Pamam) Nanopartiküller: Sentezi ve Biyomedikal Uygulamaları

Research Article

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ABSTRACT

PAMAM dendrimers are a novel class of spherical, well-designed branching polymers with interior cavities and abundant terminal groups on the surface which can form stable complexes with drugs, plasmid DNA, oligonucleotides and antibodies. Biodegradability, non-toxicity, non-immunogenicity and multifunctionality of PAMAM dendrimer are the key factors which facilitate steady increase of its application in drug delivery, gene transfection, tumor therapy, and diagnostics applications with precision and selectivity. This review deals with the major topics of PAMAM dendrimers including structure, synthesis, toxicity, surface modification, and also possible new applications of these spherical polymers.

Key Words
PAMAM dendrimer, Drug delivery, Gene transfection, Tumor therapy.

ÖZET

PAMAM dendrimerler, bir merkezden büyüyen çok düzgün bir yapıya sahip, çok dallı ve küresel üç boyutlu moleküllerdir. İç yüzeylerinde bulunan boşluklar ve dış yüzeylerinde yeralan çok sayıda terminal grup sayesinde plazmid DNA, oligonükleotidler, antikorlar ve çeşitli ilaçlarla stabil kompleksler oluşturabilirler. PAMAM dendrimerlerin toksik ve immunojenik özellik taşımamaları, bunun yanısıra biyobozunur ve çok fonksiyonlu olmaları; ilaç taşıma, gen transfeksiyonu , tümör terapisi ve tanısal uygulamalarda çok önemli bir role sahip olmalarını sağlamıştır. Bu derleme, PAMAM dendrimerlerin yapısı, sentezi, toksisitesi, yüzey modifikasyonları ve yeni uygulama alanlarını ele almaktadır.

Anahtar Kelimeler
PAMAM dendrimer, İlaç taşıma, Gen transfeksiyonu, Tümör terapisi.

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INTRODUCTION

Dendrimers, polymers of the 21st century, are three-dimensionally symmetrical polymers characterized with a unique tree-like branching architecture and have a compact spherical geometry in solution [1, 2, 3]. The word ***"dendrimer"*** originated from two words, the Greek word ***dendron***, meaning tree, and ***meros***, meaning part [4]. They simply consist of a core attached to peripheral branches (Figure 1), with total size ranging between 2 to 10 nm [5, 6, 7, 8].

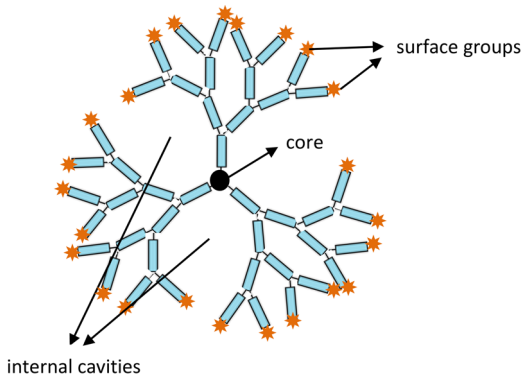

Figure 1. Dendrimer structure.

Fritz Vogtle and his co-workers introduced the first dendrimer in the 1970s. Then, in 1984, synthesizing the primary group of dendrimers was come off by Donald A. Tomalia [9, 10]. Over the past three decades, generation of multiple dendrimer families was developed in different ways and since then, their use in research, treatment of disease, and even environmental [11] and industrial applications [12, 13, 14, 15, 16] are increasing progressively.

- There are various types of dendrimers;
- Poly(amidoamine-organosilicon) dendrimers (PAMAMOS)
- Poly(amidoamine) dendrimers (PAMAM)
- Poly(propylene imine) dendrimers (PPI)
- Chiral dendrimers
- Liquid crystalline dendrimers
- Tecto dendrimers
- Hybrid dendrimers
- Multilingual dendrimers
- Micellar dendrimers [6, 17, 18, 19].

In this review, we will concentrate on PAMAM dendrimer and its various characteristics. In addition, their modifications and bioapplications will be described briefly.

PAMAM dendrimers are a group of dendrimers which are first synthesized and now are commercially available. "Starburst dendrimer" is a trademark name for a sub-class of these hyperbranched polymers on a tris-aminoethylene-imine core. The name refers to the starlike pattern observed when the structure of the high-generation dendrimers of this type is viewed in two-dimensions.

PAMAM dendrimers possess various useful characteristics which make them suitable carriers for drug and gene delivery applications [20, 21, 22]. Some of the important ones are, great numbers of branches attached to the central core, their nanometer size and manageable molecular weight [23]. Therefore, these spherical, biocompatible, safe, and non-immunogenic polymers can function as highly efficient cationic polymer vectors for delivering genetic material and different drugs into cells [24]. Additional advantages of PAMAM dendrimers include their acceptable biodegradation, their controlled drug release, and minimal nonspecific blood-protein binding properties [25].

Structure of PAMAM dendrimers

In 1984, Tomalia and coworkers synthesized PAMAM dendrimers for the first time. These dendrimers are synthetic polymers which have gained special significance as carrier system for specific genes and drugs into the various types of the cells such as primary cells, with minimum cytotoxity [26].

Basically, a PAMAM dendrimer contains three architectural components: a core, an interior of shells (generations) consisting of repeating branch-cell units, and terminal functional groups (the outer shell or periphery) [27, 28, 29].

The synthesis of these highly organized and relatively monodisperse polymers starts from the central core [30]. The core selection is important, since it can affect the molecule and surface charge density.

Table 1. Approximate diameters (Gen = 0–7) of PAMAM dendrimers. Adapted from [31].

Generation	No. of NH_2 groups on the periphery	Molecular formula	MW	Hydrodynamic diameter (nm)
0	4	$C_{24}H_{52}N_{10}O_4S_2$	609	1.5
1	8	$C_{64}H_{132}N_{26}O_{12}S_2$	1,522	2.2
2	16	$C_{144}H_{292}N_{58}O_{28}S_2$	3,348	2.9
3	32	$C_{304}H_{612}N_{122}O_{60}S_2$	7,001	3.6
4	64	$C_{624}H_{1252}N_{250}O_{124}S_2$	14,307	4.5
5	128	$C_{1264}H_{2532}N_{506}O_{252}S_2$	28,918	5.4
6	256	$C_{2544}H_{5092}N_{1018}O_{508}S_2$	58,140	6.7
7	512	$C_{5104}H_{10212}N_{2042}O_{1020}S_2$	116,585	8.1

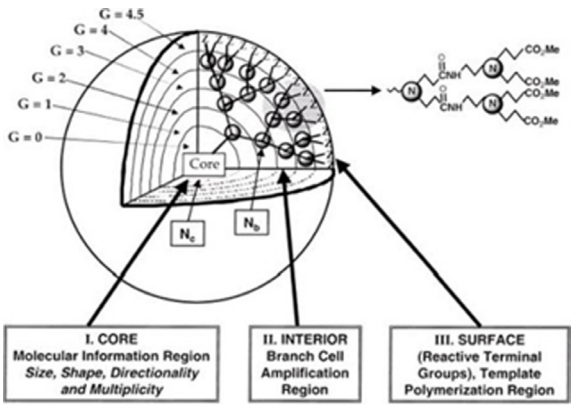

Figure 2. The core-shell structure for PAMAM dendrimer. Adapted from [31].

From this central core, "wedges" or dendrons radiate. Each layer of concentric branching units is considered as one generation and has special generation number (Figure 2).

The size of PAMAM dendrimers ranges from 10 ˚A to 130 ˚A in diameter for generation 0 (G_0) through generation 10 (G_{10}). It means that the molecule 's diameter changes approximately 1nm as each generation varies (Table 1). In detail, dendrimer diameter increases roughly linearly with generation, while the number of functional groups on the surface increases exponentially. An important consequence of this is that the distance

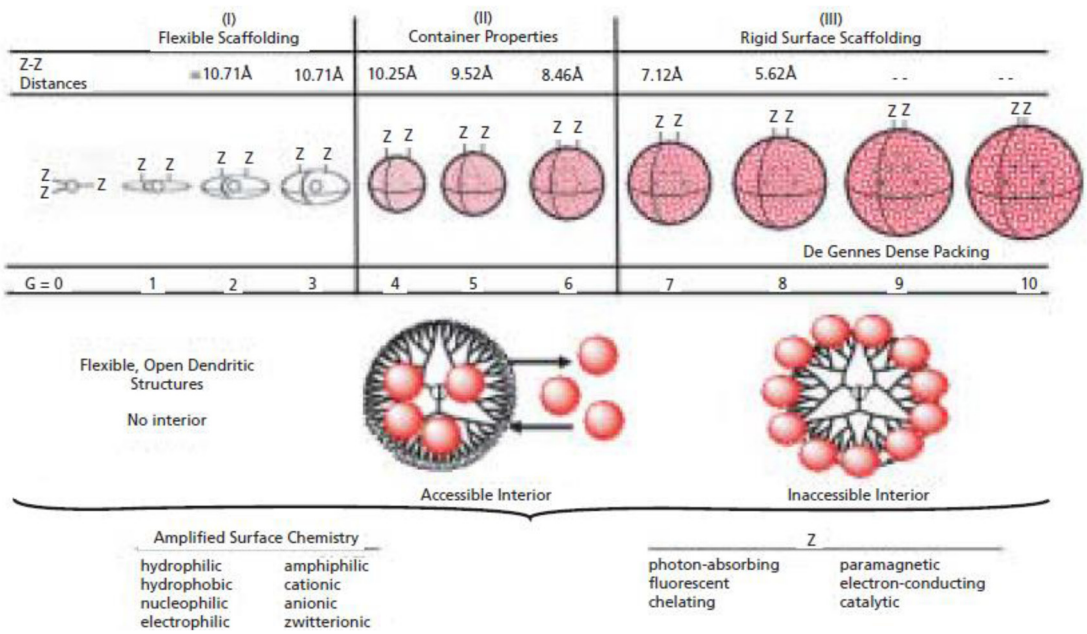

Figure 3. Periodic properties of PAMAM dendrimers as a function of generation. Adapted from [31].

between functional groups on the periphery of the dendrimer, and consequently the flexibility of peripheral groups, decreases with generation [32].

The number of generations affect the polymer 's shape. Lower generation dendrimers (G_0 to G_4) have a planar, elliptical shape, whereas at the higher generations (G_5 to G_{10}), the densely packed branches induce the polymer to form a spherical conformation. Molecular weight, size, and number of surface groups are also affected by the number of generation (Table 1). "De Gennes dense packing effect", in which the steric crowding of the branches limits developing of the higher generations (G_7-G_{10}), can lead to the defective branching structures. The produced steric hindrance starts with G_7 and reduces the synthetic yields and continues till G_{10}, and the higher generations could not synthesize because of this phenomenon (Figure 3).

There are also some other differences between lower and higher generations of PAMAM dendrimers;

1. In lower generations, the polarity is higher.
2. The higher the number of generation is, the larger the size of the cavity becomes.
3. The interaction between PAMAM dendrimers with proteins is achieved in lower generations.
4. Increase in the generation number has a direct relationship with ***in vitro*** toxicity, althought this can not be mentioned in terms of ***in vivo*** toxivity.

There are primary amine groups on the surface of PAMAM dendrimers and tertiary amine groups inside the molecule [33, 34, 35]. The primary amine groups play an essential role in binding DNA and inducing the cells to uptake the DNA, while the buried tertiary amino groups perform as proton sponge in endosomes, that cause DNA release into the cytoplasm [36]. Furthermore, the hydrophobic empty spaces in the inner region of the PAMAM dendrimers of generations higher than four, can used for encapsulating different compounds or drug delivery applications. The literature shows that high agueous solubility, unique architecture, high number of chemically versatile surface groups of PAMAM dendrimers are among the advantages which make them ideal carriers for the delivery of therapeutic agents including anticancer drugs [37].

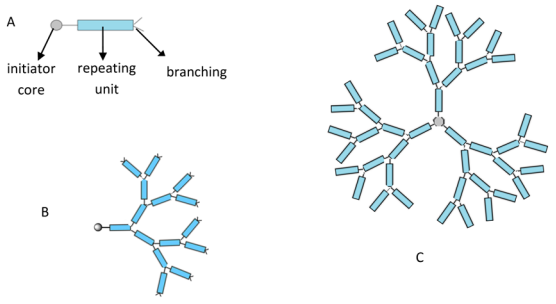

Figure 4. Structure of dendritic macromolecules: A) structural elements, B) dendron, and C) Dendrimer.

Strategies for synthesizing PAMAM dendrimers

Over the past decades, new methods are developed in order to synthesize PAMAM dendrimers [38, 39] Currently, there are four ways for synthesizing PAMAM dendrimers [40, 41], including:

Divergent synthesis

This method was introduced by Tomalia and was used for synthesizing the first class of PAMAM dendrimers. In divergent synthesis, the growth of the PAMAM dendrimer starts from a multifunctional core molecule and the reaction takes place between the core and monomer molecules containing one reactive and two dormant groups. As a result of this reaction, the first generation of PAMAM dendrimer synthesizes. Then the new periphery of the molecule activates for reactions with more monomers. Various

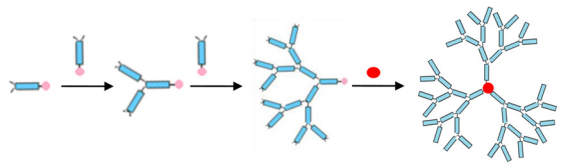

Figure 5. Convergent method for synthesis of dendrimers. The dendrimer surface is formed by reaction of the chemically active focal point (pink circles) of the branched monomer to the functional groups of another monomer. Dendrons grow by iterative coupling of monomer units to the parent dendron until the desirable dendron size is reached followed by coupling the dendron's focal point to a multifunctional initiator core (red circle) to produce the complete dendrimer.

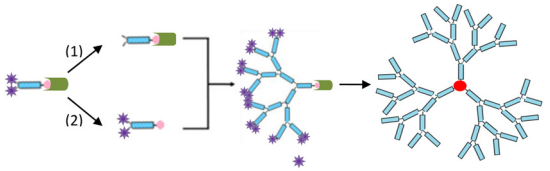

Figure 6. Convergent-divergent method for dendrimer synthesis. In this method, the branched monomer is protected at the focal point (pink circle) with green protection and at the terminal groups with purple protection. Using separate deprotection schemes, the monomer's terminal groups (1) or the focal point (2) are deprotected and coupled together to yield the dually protected dendron. Divergent dendron growth can then continue through purple deprotection and iterative monomer coupling or the complete dendrimer can be formed by green deprotection and coupling of the dendron focal point to a multifunctional core followed by purple deprotection (1).

layers of the dendrimer produce after repetition of these reactions [10, 31, 42].

The initiator core for PAMAM dendrimers is an ammonia or ethylenediamine (EDA) molecule. Ammonia has three and EDA has four possible binding sites for amidoamine repeating units. The primary amino groups are on the surface of the molecule and two new branches may be conjugated to each of them (Figure 4) [43].

Convergent synthesis

In 1990, Hawker and Frechet introduced the convergent method with the purpose of eliminating some of the obstacles of the divergent method. Compared to divergent method, in this top-down method, a dendrimer is synthesized from the surface towards the core, mostly by "one to one" coupling of monomers, thereby creating dendritic segments, dendrons, of increasing size as the synthesis progresses. The final part of the convergent synthesis finishes at the core, where two or more dendritic segments (dendrons) are joined together, creating the dendrimer. Thus, the convergent strategy has generally an inverse propagation compared to the divergent strategy (Figure 5) [44, 45].

Combined convergent-divergent synthesis

A hybrid convergent-divergent synthesis method called "double exponential growth" was described by Kawaguchi and his coworkers. In this method, orthogonally protected branched monomers for accelerating dendrimer synthesis are used with protecting groups which are stable during cleavage of the opposing functionality (Figure 6) [46].

Click Synthesis

Click dendrimers have evolved in 2004 as an easy and practical method. Among the various click reactions, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) rapidly appeared as the most useful one [47]. The click chemistry refers to Cu-catalyzed cycloaddition reaction of an alkyne and an azide to form a 1,2,3-triazole ring. This reaction is clearly a breakthrough in the synthesis of dendrimers and dendritic and polymer materials. The click dendrimers provide a bridge between dendritic architectures and nanomaterials [48].

Surface modification of PAMAM dendrimers

In recent years, there has been an increasing number of studies on dendrimers application as drug carriers, transfection agents, or magnetic resonance imaging agents. A serious drawback of dendrimers is their toxicity, mainly due to the surface positive charge of the PAMAM dendrimers. One of the ways for reducing the toxicity of PAMAM dendrimers is modification of these surface groups. In other words, toxicity reduction and biocompatibility improvement are the results of the appropriate surface modifications of PAMAM dendrimers [49, 50].

As an alternative for decreasing cytotoxicity of PAMAM dendrimers, amine acetylation can be pointed out. Amine acetylation of PAMAM dendrimers can lead to an increase in water-solubility, and this quality is essential for biomedical uses that need solubility in aqueous solutions [51]. Conjugation with poly(ethylene glycol) (PEG) chains, folic acid, and biotin has been considered as other methods for reducing the toxicity and increasing the biocompatibility and the circulation time of dendrimers in the body [52, 53, 54].

Pharmaceutical applications of PAMAM dendrimers

Unparalleled molecular uniformity, multifunctional surface and presence of internal cavities, make

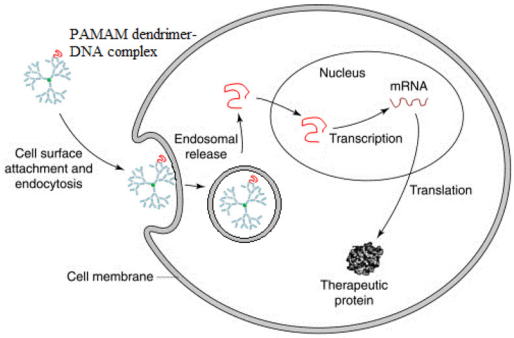

Figure 7. Gene transfection of PAMAM dendrimer.

PAMAM dendrimers particularly well-suited to possible pharmaceutical applications [10]. Therefore, PAMAM dendrimers are selected as appropriate candidates in order to use in a variety of high technologies [55] which are as follows:

Drug delivery

The unique properties of PAMAM dendrimers, namely their special three-dimensional architechture, the large number of functional groups present on their surface, their great monodispersity and their high water solubility make them excellent candidates for evaluation as drug carriers, since these characteristics can facilitate and modulate the delivery process. The host-guest interactions between PAMAM dendrimers and drugs are dependent on the protonation ability (p*K*a), steric hindrance, size, hydrophobicity, and numbers of binding sites of the drug molecules [56, 57, 58]. There are two possibilities for drug molecules to be carried by PAMAM dendrimers, either loading in the interior of the dendrimers, or attaching to the surface groups. In other words, PAMAM dendrimers can function as drug carriers either by encapsulating drugs within the dendritic structure, or by interacting with drugs at their terminal functional groups via electrostatic or covalent bonds (prodrug) [10, 59, 60,61]

Gene therapy

Gene therapy is a new technology providing a promising approach to eradicate genetic disease. The potential of dendrimers as gene delivery agents has been explored for several years [40, 62]. In 1993, Haensler and Szoka published the first report of the use of PAMAM dendrimers for gene transfection. They found that PAMAM dendrimers mediated the high-efficiency transfection of DNA into a variety of cultured mammalian cells, and that the transfection was a function of both the dendrimer–DNA ratio and the diameter of the dendrimers [55, 60]. Various reports indicated that PAMAM dendrimer could successfully introduce a reporter gene in different cells both in vitro and in vivo. In other words, they can act as non-viral transfection agents in gene therapy. As their net charge is positive under physiological conditions, it is possible to form a complex with DNA [63]. Then, this complex can bind to negatively charged surface molecules on cell membrane. Followed by internalization into the cells via non-specific endocytosis, lysosomal degradation of the PAMAM dendrimer-DNA complexes takes place. After releasing the targeting gene, it enters into the nucleus to play a role in gene therapy (Figure 7)[64].

Dendrimer as solubility enhancers

There are many substances, which have a strong therapeutic activity but due to their lack of solubility in pharmaceutically acceptable solvents, they have not been used for therapeutic purposes. Water soluble dendrimers are capable of binding and solubilizing small acidic hydrophobic molecules with antifungal or antibacterial properties. In other words, they solve poorly soluble drugs by encapsulating them within the dendritic structure [65, 66, 67]. In comparison with interior encapsulation, surface ionic interaction was found to be the major factor on the solubilization behavior of PAMAM dendrimer [68]. Different generations (G_2-G_4) of PAMAM dendrimers have the potential to significantly enhance the solubility of drugs. The higher solubility may contribute to a higher drug bioavailability [69].

Cellular delivery using PAMAM dendrimer carriers

In recent years, various studies indicated the application of PAMAM dendrimers to deliver drugs efficiently into cells, and even target intracellular compartments. These dendrimers can also play an essential role as carriers to cross biological barriers, such as the gastrointestinal tract [8] and blood–brain barrier [70], therefore they can use

as vehicles to transport several water-insoluble drugs like ibuprofen [71], indomethacin [72], flurbiprofen [73], methotrexate [74], niclosamide [75], doxorubicin [76], and many others [77, 78] into cell.

PAMAM dendrimers in photodynamic therapy

Photodynamic therapy (PDT) has emerged as a new noninvasive clinical treatment of superficial tumors in recent years [79]. In this method, certain therapeutic molecules called photosensitizers are administrated usually through intravenous route and they are accumulated in malignant tissue. Then, activation of these photosensitizers by light of appropriate wavelength is performed, and they pass on their excess energy to nearby molecular oxygen to form reactive oxygen species such as singlet oxygen ($^1O_2$) and free radicals, which are toxic to cells and tissues [80]. For an effective photodynamic effect, several ideal properties of photosensitizers are required. From the chemical point of view, the materials should be pure and have a high quantum yield of singlet oxygen generation. From the biological point of view, it should possess no dark toxicity and have high solubility in an aqueous medium for the easy administration. High tumor localization and long wavelength absorption are also very important for effective medical treatment [81].

PAMAM dendrimers in photothermal therapy

PAMAM dendrimer encapsulated gold nanoparticles have been synthesized that strongly absorb light in the near-infrared region, facilitating deep optical penetration into tissues, generating a localized lethal dose of heat at the site of tumor. Over the past few years, PAMAM dendrimer-encapsulated gold nanoparticles have been prepared and identified for their potential use towards the photothermal treatment of malignant tissue [82].

Neutron capture therapy

Neutron capture therapy (NCT) is an appropriate method for the treatment of intractable tumors such as brain tumors. NCT using ^{10}B, Boron neutron capture therapy, has demonstrated efficacy in the treatment of tumors [83].

Boron neutron capture therapy (BNCT) is a new method based on selective uptake of sufficient amounts of a stable isotope, ^{10}B, by tumor cells, followed by irradiation with low energy thermal neutrons. The resulting nuclear capture and fission reactions yield α-particles and ^7Li nuclei, which have high linear energy transfer. These particles have limited path lengths in tissue (< 10 μm) and essentially deposit their energy within these cells and thus their toxicity is limited to cells that have internalized ^{10}B. The efficacy of BNCT depends on the selective delivery of a relatively high amount of ^{10}B to tumor cells while sparing adjacent normal cells. It has been estimated that ~15 μg of ^{10}B must be delivered to each gram of tumor in order to sustain lethal tumor cell damage [84, 85, 86, 87, 88].

An alternative to BNCT is the Gadolinium-based (Gd) neutron capture therapy that has been investigated due to Gd's high neutron absorbency properties, but has rarely been used as it is deemed too difficult to achieve therapeutic doses intravenously [82].

Diagnosis and imaging

In recent years, PAMAM dendrimers have become an exciting class of polymeric platforms for assembly of multifunctional nanomaterials undergoing pre-clinical development as diagnostics vehicles [89]. Biocompatible PAMAM dendrimers can carry imaging probes such as magnetic resonance imaging (MRI) contrast, fluorescent dye, and radionuclide to detect cancerous cells, and simultaneously deliver anticancer drugs directly to the targeted disease. Targeted drug delivery using PAMAM dendrimers as a multifunctional scaffold can improve the therapeutic effect of traditional cancer treatment, avoid the adverse effects of the drugs, and develop a simplified drug administration schedule with improved safety [90]. PAMAM dendrimer chemistry and nanotechnology allow these PAMAM dendrimer-based drug carrier systems to be administered in different routes such as oral, injectable, transdermal, ocular, nasal, colon, rectal, and pulmonary delivery systems [26, 91, 93].

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