Nano-Biomaterials for Retinal Regeneration

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Contributor: Nikhlesh Singh

Nanoscience and nanotechnology have revolutionized key areas of environmental sciences, including biological and physical sciences. Nanoscience is useful in interconnecting these sciences to find new hybrid avenues targeted at improving daily life. Pharmaceuticals, regenerative medicine, and stem cell research are among the prominent segments of biological sciences that will be improved by nanostructure innovations. Nanoparticles, nanowires, hybrid nanostructures, and nanoscaffolds, that have been useful in mice for ocular tissue engineering and regeneration.

nanoparticles nanodisks scaffolds nano-biomaterials and retina nanoscaffolds and retinal regeneration nanoparticles and retinal regeneration

1. Introduction

To overcome the limitations of conventional eye drops and of intraocular invasive injections, several ophthalmic formulations have been proposed, such as drug-loaded nanoparticles/nanocarriers. Nanoparticles, which are submicron-sized particles ranging from 10 to 1000 nm, can provide a versatile platform for drug delivery. Drugs can be loaded into such nanoparticles by attachment to the matrix, or the drug can be dissolved, encapsulated, or entrapped within their nanomorphologies. In various stages of clinical studies, the Food and Drug Administration (FDA) has approved nearly 250 nanomaterial-based medical products [1]. With recent advancements, nanomedicine approaches to the regeneration of tissues have been particularly focused on using certified functional nanomaterials. These engineered nanomaterials not only deliver cells and tissues but also monitor tissue regeneration processes in real time, thereby improving the overall therapeutic efficiency. The compatibility of biological organs with various nanomaterials, such as nanoparticles (NPs), nanowires (NWs), and hybrid nanostructures, has enhanced the probability of their use in biomedical applications, especially in retinal regeneration (**Figure 1**) [2][3][4][5][6][7]. Among these, nanoparticles such as gold NPs (AuNPs) and magnetic iron oxide nanoparticles (MIONPs) are widely used in preclinical and clinical settings due to their well-established imaging and therapeutic properties [8][9]. Furthermore, because of their physical and chemical properties, nanoparticles have recently been introduced as contrast enhancement agents for many imaging modalities such as MRI [10][11][12][13][14], fluorescence imaging [15], photoacoustic imaging [16], ultrasound imaging, and computed tomography (CT) [17][18][19][20][21][22][23][24]. In recent years, modified nanoparticles have been in high demand for their use in clinical practices for in vitro metabolic assays. In this context, studies have shown that gold nanoparticles deposited on the plasmonic chip and a porous silica-based plasmonic nanoreactor are useful for the metabolic analysis of biofluids [25][26]. Some studies have used nano-biomaterials to treat antibiotic-resistant

bacterial infections [27]. Furthermore, the use of platinum nanoreactor, polymer@Ag-assisted, and bimetallic alloy-based laser desorption/ionization mass spectrometry showed its usefulness for metabolic fingerprinting and disease diagnosis [28][29][30].

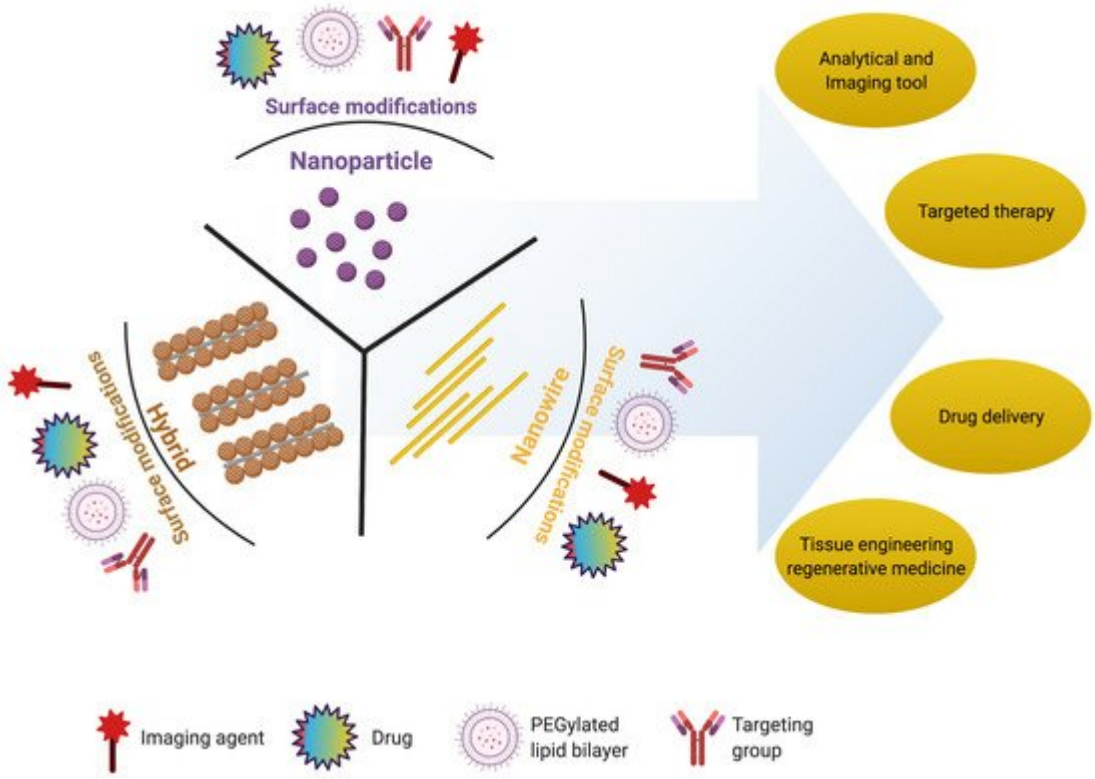

Figure 1. Schematic representation of multifunctional nanostructures: nanoparticle (NP), nanowire (NW), and hybrid with various applications in biomedical science. These nanostructures can be surface modified with drugs (incorporated or conjugated to the surface), a PEGylated lipid bilayer (to improve solubility and decrease immunogenicity), targeting groups (to improve nanostructures' circulation, effectiveness, and selectivity), and imaging agents (e.g., fluorescent dyes used as reporter molecules and employed as tracking or contrast agents).

2. Nanomaterials for Retinal Regeneration

In the present section, we will discuss the importance of nanoparticles, nanowires, and hybrid nanostructures in retinal regeneration, summarized in **Table 1**.

Table 1. Details of various nanostructures and their morphologies for targeting specific tissues or cells for retinal regeneration.

Nanostructure	Nanomaterial	Size Range (nm)	Target Tissue/Cells	Ref.
Nanoparticles	Gold (Au) (diameter)	3–5	Choroidal and retinal endothelial cells	[31]
		10–12	Retina of rabbit	[32]

Nanostructure	Nanomaterial	Size Range (nm)	Target Tissue/Cells	Ref.
		10–20	Photoreceptor precursor transplantation	[33]
		80	Retinal cells	[34]
		20–80	Nucleus and mitochondria of retinal cells	[35]
		5–20	Blood–retinal barrier	[33] [36] [37]
	Gold (Au) nanodisk	Thickness: 20 Diameter: 160	Retina	[36]
	Silver (Ag) (diameter)	20–80	Bovine retinal endothelial cells	[38]
		40–50	Porcine retinal endothelial cells	[39]
	Superparamagnetic iron oxide nanoparticles	Diameter: 5–20	Retina	[40]
	Magnetite	10	Retina and cells	[41] [42]
	Poly (ε-caprolactone) (PCL) membranes	Length: 2500	Implantation into subretinal space	[43]
NWs	Gallium phosphide (GaP)	Length: 500–4000	Retinal cells	[44]
	n-type silicon	Length: 4400	Retinal cells	[45]
	Gold (Au) nanorods	Thickness: 10–35	Retinal cells and photoreceptors	[46]
Hybrid nanostructure	Gold NPs coated over titania (TiO_2) NWs	Au NP diameter: 5–15 TiO_2 NW length: 2000	Artificial photoreceptors	[43] [47] [48] [49] [50]

Nanostructure	Nanomaterial	Size Range (nm)	Target Tissue/Cells	Ref.
	Gallium phosphide (GaP) rod and cone	Length: 20–2500	Ganglion cells, and bipolar cells	[44]
	Gold NPs coated over silicon NWs	Au NP diameter: 5–10 NW length: 500–2500	Artificial photoreceptors	[51] [52]
	Thin film functionalized with the NPs	Diameter: 5–50	Photoreceptors	[53] [54]
	p–n junction silicon NWs	NW length: 10–120	Membranes of live bipolar cells	[55]
	Au-coated carbon nanotube (Au-CNT)	Au NP diameter: 5–20 CNT length: 500–2500	Subretinal space of mice	[56]
	Iridium oxide (IrOx) combined with reduced graphene oxide	IrOx diameter: 2–25 CNT length: 2–2500	Subretinal implant into live mice	[57]
	Iridium oxide (IrOx) coated with CNT	IrOx diameter: 5–25 CNT length: 500–2500	Retinal cells/tissues	[41] [58] [59] [60] [61]
	Core–shell-structured β-NaYF4:20%Yb, 2%Er@β-NaYF4 nanoparticles	Diameter: 30–40	Subretinal space of mice	[4]
Nanoscaffolds	Natural polymer: gelatin, fibrin, chitosan, laminin, and hyaluronic acid	Diameter/porosity: 100–200	Extracellular matrix and cell attachment	[62] [63] [64] [65] [66] [67]
	Synthetic polymer: poly (lactic-*co*-glycolic acid) (PLGA), poly (ε-caprolactone) (PCL), poly (L-lactic acid) (PLA), polyimide, and poly (l-lactide-co-ε-caprolactone)	Diameter/porosity: 50–500	RPE, biological activity, extracellular matrix, and cell attachment	[68] [69] [70] [71]
	Biohybrid: nanofibers of Bruch's membrane	Diameter/porosity: 100–200	RPE and biological activity	[72]

Mammalian near-infrared image vision through injectable and self-powered retinal nanoantennae. Cell 2019, 177, 243–255.

2.1. Nanoparticles

Nanoparticle-based gene and drug delivery to retinal cells has been harnessed to treat various eye diseases [33][73][74][75][76][77][78][79][80]. The various transport mechanisms that nanoparticles employ to cross the blood–retinal barrier are shown in **Figure 2**. Nanoparticles absorb or scatter light at specific frequencies/wavelengths as a function of their physical and chemical characteristics. These properties of nanoparticles are useful for bioimaging and to treat cancer by using near-infrared-triggered photothermic therapy (PTT) [81]. Due to the low absorption coefficients of hemoglobin and water, the penetration of near-infrared (650–900 nm) rays in tissues is very high, allowing the use of near-infrared rays for nanoparticle stimulation without damaging the tissue [82]. Gold-nanoparticle-based intravitreal injection is used for retinal imaging and for the inhibition of retinal neovascularization to treat macular degeneration [78][79][80].

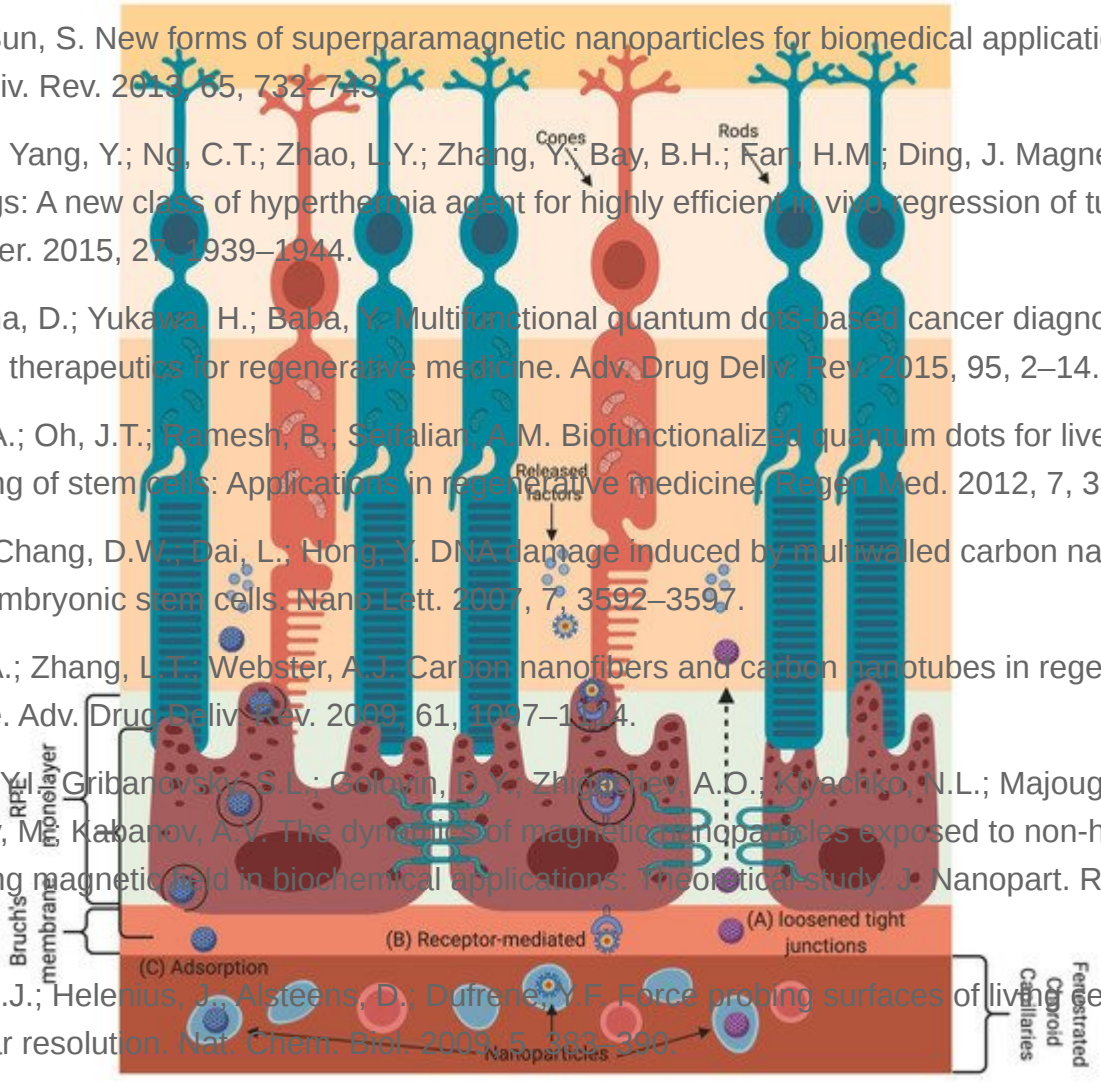

Figure 2. Transport mechanisms for delivering nanoparticles (NPs) into the blood–retinal barrier (BRB). The BRB is exceedingly selective and has unambiguous transport mechanisms allowing a close control of molecules/cells that enter the retina. Loosening of tight junctions (TJs) either due to the presence of a surfactant in NPs or by BRB impairment due to pathological conditions allows the movement of NPs through the BRB. (**A**) NPs' admittance into

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the ligand is through receptor-mediated transcytosis. (**B**) Then, NPs interact with respective receptors on the endothelial cell surface, which leads to plasma membrane invaginations, vesicle formation, and, therefore, the release of the NPs at the other side of the membrane. (**C**) NPs coated with chitosan or other polysaccharides can cross the BRB by adsorptive transcytosis.

2.2. Nanowires

Engineered nanostructural materials are essential for the development of advanced retinal applications. Among them, nanowires have been reported for retinal applications in recent years [48]. It has also been demonstrated that the structure and morphology of nanowires are similar to those of photoreceptors, and the photoabsorption and charge separation properties of nanowires are comparable to those of photodetectors or solar cells [49]. The gold-nanoparticle-decorated titania (Au-TiO_2) nanowire acts as an artificial photoreceptor that restores the light responses in a photoreceptor-degenerated retina. The use of nanowires with poly (ε-caprolactone) (PCL) scaffolds for the delivery of retinal progenitor cells resulted in increased differentiation and migration of these cells into both degenerated and normal retinas [43][50]. Nanowires made of gallium phosphide have been shown to support the long-term survival of photoreceptors (rods and cones), ganglion cells, and bipolar cells [44].

Nanowires not only sense the incoming light but also transfer electrical signals within rod and cone cells [54]. To create nanowires that can transfer electrical signals, researchers have used n-type and p-type silicon materials. These silicon materials sense light and transform it into electrical signals [3][83]. Furthermore, the one-dimensional morphology of silicon nanowires is better suited to sense light and convey the signals to the various retinal layers to correct the visual impairment [3]. The incorporation of *n*-type and *p*-type silicon in nanowires has made it possible for the nanowires to convert light signals to electrical signals and then transfer them into the membranes of live bipolar cells for vision recovery [84].

2.3. Hybrid Nanostructures

A vast range of nanomaterials and nanostructures have been explored as neural interfaces in retinal physiology; still, no single material has been successful in mimicking the biological, mechanical, and electrical properties of the retina. In recent years, many hybrid approaches have been designed to explore the merits of many materials while at the same time suppressing their demerits. Recently, Tang et al. demonstrated that artificial photoreceptors made of gold-nanoparticle-decorated titania (Au–TiO_2) nanowire arrays were able to absorb light, generate photovoltage, and process visual information in a photoreceptor-degenerated retina [3]. Not only is nanowire arrays' rough morphology useful for their association with cultured neurons, but they are also biocompatible or (photo)chemically stable for over 2 months when used as a subretinal implant in mice [55]. The use of a gold coating on carbon nanotubes (Au-CNTs) further enhanced their surface area and electrical and mechanical adhesion [85]. Iridium oxide-carbon nanotube hybrids (IrOx-CNT) were reported to have a high effective surface area and much higher charge storage capacities compared to pure iridium oxide [56]. Furthermore, the hybrid coatings formed by combining iridium oxide with reduced graphene oxide or graphene oxide exhibited 10% higher charge storage

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capacities than those of pure iridium oxide, and iridium oxide–carbon nanotube hybrids, indicating superior electrochemical stability [41][57][58][59][60].

2.4. Nanoscaffolds

Nanoscaffolds are self-assembled or electrospun nanofibers made up of synthetic or natural polymers. Nanoscaffolds provide a microenvironment for cellular signaling that influences the proliferation, migration, and differentiation of various cells [86].

These scaffolds are made up of natural nanofibers/polymers. Collagen I is a major component of retinal pigment epithelial cells, and therefore, ultrathin collagen I membranes were used to design natural nanoscaffolds. These membranes were stable for 10 weeks and degraded within 24 weeks. Other natural polymers used for retinal regeneration studies include gelatin [62], fibrin [63], chitosan [64], laminin [65], and hyaluronic acid [66]. The chemistry of natural nanoscaffolds makes them more suitable for cell attachment and biological activity [67].

Synthetic nanoscaffolds are easier to design, and their physical properties can more easily be controlled to mimic the extracellular matrix compared to natural polymers [79]. Poly (lactic-*co*-glycolic acid) (PLGA) [68], poly (ε-caprolactone) (PCL) [69], poly (L-lactic acid) (PLA) [68][70], polyimide [71], and poly (l-lactide-co-ε-caprolactone) [68] are commonly used synthetic polymers.

Biohybrid nanoscaffolds are made by combining both natural and synthetic nanofibers to make composite scaffolds. Biohybrid nanoscaffolds have the appearance and protein composition of a natural nanoscaffold and the design of synthetic nanoscaffolds. Studies have shown that biohybrid nanoscaffolds are well tolerated without any adverse inflammatory reaction in the retina [72], but there is a need to characterize the various components of biohybrid nanoscaffolds for their reproducibility.

3. Studies on the Application of Nano-Biomaterials for Retinal Regeneration

Retinal transplantation is considered a limiting factor for the treatment of blinding diseases due to the complex neural network [87]. Therefore, tissue regeneration using scaffolds with acceptable biocompatibility is a recent, more promising approach to repair damaged tissues or organs. Scaffolds likely simulate the extracellular matrix (ECM) and thus have the capability to support cell migration, adhesion, and morphology in the regeneration of the retina [87][88][89]. Nanomaterials with unique properties and a hierarchical architecture have been developed for multidisciplinary applications and have the capability to significantly advance the field of tissue/organ regeneration. As a result, various investigators have developed nanomaterials with better biocompatibility, electroconductivity, and capacitance to enhance the efficiency of tissue regeneration [90][91][92][93][94]. Various in vitro, in vivo, and therapeutic studies have highlighted the importance of nanostructures in retinal regeneration, and a summary is presented in **Table 2**.

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Table 2. In vitro and in vivo studies of various nanomaterials and synthesis used to promote retinal

Analysis	Nanomaterial	Form	Size (nm)	Cell Response	Ref.
In vitro	Poly (ε-caprolactone) (PCL)	NWs	Length: 2500	↑ expression of PKC and recoverin in RPCs; cells undergo differentiation	[43]
	Gallium phosphide (GaP)	NWs	Length: 500–4000	Extended growth of retinal cells	[50]
	n-type silicon	NWs	Length: 440	Long-term and dense growth of mouse retinal cells	[95]
	Gold (Au)	Nanoparticle	Diameter: 5–100	ARPE-19 cells undergo apoptosis upon AuNP internalization	[66]
			Diameter: 10–12	Gold nanoparticles inhibit proliferation of ARPE-19 cells; no cytotoxicity	[5]
			Diameter: 80	Highly viable mesenchymal stem cells undergo differentiation and secrete various trophic factors	[4]
	Gold (Au), silver (Ag)	Nanoparticle	Diameter: 20–80	Increase uptake into retinal cells; ↑ apoptosis, oxidative stress, and microglia activation	[47]
	Gold (Au)	Nanodisk	Diameter: 160	Inhibition of in vitro angiogenesis without cellular toxicity of HRMECs	[45]
	Hybrid nanoscaffolds	Combination of *Antheraea pernyi* silk fibroin (RWSF), PCL, and gelatin	Diameter/porosity: 90–210	Increased expression of RPE marker genes (CRALBP, PEDF, VEGF, MITF, and PMEL 17 among others)	[72]
In vivo	Poly (ε-caprolactone) (PCL) membranes	NWs	Length: 2500	Successful implantation into subretinal space with limited tissue disruption and no inflammation	[43]

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Analysis	Nanomaterial	Form	Size (nm)	Cell Response	Ref.
	Gold (Au), titania (TiO_2)	Au nanoparticle coated TiO_2 NWs	AuNPs diameter: 5–15, TiO_2 NW length: 2000	AuNP-decorated TiO_2 NW arrays restore light-sensitive visual responses in degenerated photoreceptors	[3]
	Gold (Au)	Nanodisk	Diameter: 160	Intravitreal injection attenuates neovascularization in mouse model of oxygen-induced retinopathy	[45]
	Gold (Au)	Nanoparticle	Diameter: 20–100	Intravitreal injection of gold nanoparticles passed through the blood–retinal barrier with no structural abnormality or cell death	[80]
	Gold (Au)	Nano-gold	Not reported	No retinal or optic nerve toxicity by intravitreal injection of nano-gold	[32] [80]
	Gold (Au), poly (strenesulfate)	Poly (strenesulfate) or anti-CD90.2 antibody-coated Au nanorods (PSS-AuNRs)	Not reported	Intravitreal injection obscured the retinal signal and induced ocular inflammation	[46]
	Nanoscaffolds	Nanofibrous porous membrane	Diameter/porosity: 680	Bruch's membrane thickness changes with aging, and it correlates with RPE function	[72]
Therapeutic	Gold (Au)	Nanoparticles	Diameter: 20	AuNP-labeled photoreceptor precursor transplantation provides high-resolution long-term tracking and cell survival with no toxic effects on retina or cells	[80] [96]
	Core–shell-structured β-NaYF4:20%Yb, 2%Er@β-NaYF4	Nanoparticle (core–shell-structured upconversion nanoparticles (UCNPs))	Diameter: 35–40	Retinal pbUCNP injection extends the visual spectrum to the near infra-red range in mice	[4]

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an artificial Bruch's membrane made of nanofibers for engineering of retinal pigment epithelium cell monolayers. Acta Biomater. 2013, 9, 9414–9422.

Analysis	Nanomaterial	Form	Size (nm)	Cell Response	Ref.
	Synthetic nanoscaffolds	Nanofibrous scaffolds	Diameter/porosity: 100–200	Used as a cell replacement therapy	[86]

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