Journal of Controlled Release 145 (2010) 76–90

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Silver nano — A trove for retinal therapies

Kalimuthu Kalishwaralal [1], Selvaraj BarathManiKanth [1], Sureshbabu Ram Kumar Pandian, Venkatraman Deepak, Sangiliyandi Gurunathan *

Department of Biotechnology, Division of Molecular and Cellular Biology, Kalasalingam University, Anand Nagar, Krishnankoil-626190, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 21 January 2010
Accepted 24 March 2010
Available online 29 March 2010

Keywords:
Silver nanoparticles
Drug delivery
Retinopathy
RGD peptide
Controlled release

ABSTRACT

Pathological retinal angiogenesis (neovascularization) is one of the most feared complications among retinal diseases, leading to visual impairment and irreversible blindness. Recent findings made by us on therapeutic applications of biologically synthesized silver nanoparticles (AgNPs) against VEGF induced retinal endothelial cells, elucidates the effectual inhibitory activities of AgNPs over the downstream signaling pathways (Src and AKT/PI3K) leading to retinal angiogenesis. The current review focuses on the imperative role of VEGF induced angiogenesis in the development of retinal neovascularization and despite the fact that several VEGF targeting ocular drugs are available; the review examines the need for a cost economic alternative, thereby suggesting the role of AgNPs as an emerging economic ocular drug for retinal therapies. The current technologies available for the development of targeted and controlled release of drugs is being discussed and a model has been proposed for the amenable targeting mechanism, by which Poly gamma glutamic acid (PGA) capsulated AgNPs conjugated to cyclic RGD peptides carry out a sustained controlled release specifically targeting the neovascularization cells and induce apoptosis unaffecting the normal retinal cells. These constructs consequently affirm the futuristic application of silver nanoparticles as a boon to ocular therapies.

Contents

* Corresponding author. Department of Biotechnology, Kalasalingam University, Kalasalingam Academy of Research and Education, Anand Nagar, Krishnankoil-626 190, Tamil Nadu, India. Tel.: +91 4563 289042; fax: +91 4563 289322.
E-mail address: lvsangs@yahoo.com (S. Gurunathan).
[1] These authors contributed equally.

0168-3659/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2010.03.022

1. Introduction

Visual loss is one of the most feared complications of human diseases other than death [1]. Pathological retinal angiogenesis (retinal revascularization) is the leading cause of severe vision loss and irreversible blindness in developed countries, affecting people of all ages [2]. Despite tissue specific aspects, retinal revascularization shares many common features with other pathological angiogenic conditions, such as in tumor genesis, rheumatoid arthritis and atherosclerosis [3]. In developed countries, the primary causes of visual loss are age-related macular degeneration in the elderly and diabetic retinopathy, in working-age persons [1]. Hence gaining the ability to control retinal angiogenesis does not only benefit clinical therapy, but also has an enormous scientific appeal.

Ophthalmic complications of diabetes include corneal abnormalities, glaucoma, iris neovascularization, cataracts, and neuropathies [4]. The major retinal problems leading to diabetes-related vision loss include diabetic macular edema that occurs due to the leaking of fluid from blood vessels within the macula [5] and the other is the complications from abnormal retinal blood vessel growth, i.e. angiogenesis terming to the growth of new blood vessels from the pre-existing retinal circulation into the vitreous humor [6]. Angiogenesis, the formation of new blood vessels is essential for the growth and progression of tumors. Secondary to angiogenesis, increased retinal blood flow is of pathogenic importance in the progression of diabetic retinopathy [7]. Thus visual loss primarily occurs from either proliferation of new retinal vessels (proliferative diabetic retinopathy) or increased permeability of retinal vessels (diabetic macular degeneration). Initially there is capillary loss in the mid-peripheral and peripheral retina and the resultant leads to stimulation of retinal hypoxia thereby over expressing pro-angiogenic growth factors, with vascular endothelial growth factor (VEGF) and the growth hormone/insulin-like growth hormone-1 axis being implicated in the resultant angiogenic response [8]. The vascular endothelial growth factor [VEGF) is the major angiogenic factor in the retina that promotes neovascularization and vascular leakage [9–11]. The balance between VEGF and angiogenic inhibitors may determine the proliferation of angiogenesis in diabetic retinopathy. Thus the lifetime risk for diabetes to develop macular edema is about 10%, whereas the condition is closely associated with the degree of diabetic retinopathy [12].

The design of ocular drug delivery systems is undergoing a gradual transition from an empirical to a rational base. Interest in the broad area of ocular drug delivery has increased in recent years due to an increased understanding of a number of ocular physiological processes and pathological conditions, including aqueous humor dynamics, inflammation, corneal wound healing, and cataract genesis [13]. Thus, ophthalmic scientists are eagerly searching for new therapeutic strategies that can selectively target pathological neovascularization and other retinal complications.

Nanoparticles have become a part of our daily life, in the form of cosmetics, electronics, computer science, biosensors and various others. In the last few years, several pharmacological companies won approval from the Food and Drug Administration (FDA) in the US for the use and development of nanotechnology-based drugs. They have attracted considerable attention in recent years, owing to their various therapeutic applications [14]. The potential benefits of nanomaterials in biomedical and industrial applications for human health and environment have been proved with respect to their various physico-chemical and biological activities [15]. The global market for medical nanotechnology has been expected to reach more than $3 billion within the next five years [16]. Over these few years the use of nanoparticles has given remarkable breakthroughs to the field of nanomedicine and has altered the foundations of disease diagnosis, treatment and prevention based on their properties. Furthermore, there is a wide array of fascinating nanoparticulate technologies capable of targeting different cells and extra cellular elements in the body to deliver drugs, genetic materials, and diagnostic agents specifically to these locations [17–20]. Silver nanoparticles (AgNPs) are emerging as an arch product from the field of nanotechnology that have gained interest over the recent years as a nanomedicine due to their distinctive properties and vivid therapeutic applications [21].

This current review focuses on the crucial role of angiogenesis in the development of diabetic retinopathy through vascular endothelial growth factor (VEGF) as a foremost growth factor; the availability and issues of the ocular drugs for their treatment and resolves the effective role of silver nanotechnology in ophthalmological therapies towards VEGF induced retinal angiogenesis It also explores the indispensable role of RGD and Poly gamma glutamic acid in developing a targeted and controlled release system for drug delivery. A proposed model for the targeted and controlled release of silver nanoparticles as a drug in ocular neovascularization using polymer encapsulated silver nanoparticles conjugated to RGD peptide is given, thereby stating AgNPs futuristic applications as an ocular drug.

2. Angiogenesis mediates diabetic retinopathy

2.1. Angiogenesis paves way to complications since the ancient — an overview

Angiogenesis is a significant phenomenon involved in normal growth and wound healing processes in the system. An imbalance of the growth factors involved in this process, however causes the acceleration of several diseases including malignance, ocular, and inflammatory diseases. In 1971, the first hypothesis about angiogenesis was given, that tumour growth is angiogenesis dependent and that inhibition of angiogenesis could be therapeutic. The term anti-angiogenesis was introduced during these researches that meant the prevention of new vessel sprout from being recruited by a tumour. This hypothesis predicted that tumours would be unable to grow beyond a microscopic size of 1–2 mm^3 without continuous recruitment of new capillary blood vessels. The above proposed concept is now widely accepted w.r.t. the supporting data's from experimental studies and clinical observations carried out over the intervening years [22,23]. Evidences for the dependence of tumour growth on neovascularization state the role of angiogenesis in tumour formation. Tumour growth after vascularisation was at an exponential rate when compared to the tumour growth in the avascular cornea that proceeded slowly at a linear rate [24]. Tumours grown in the vitreous of the rabbit eye remained viable but attained diameters of less than 0.50 mm for as long as 100 days. Once such tumour reached the retinal surface, it became neovascularized and within 2 weeks an increase in volume of about 19,000-fold over the avascular tumour was observed [25]. In transgenic mice that developed carcinomas of the β cells in the pancreatic islets, large tumours arose only from a subset of preneoplastic hyperplastic islets that became vascularised [26]. The metastases were suppressed when a primary tumour was

implanted and allowed to grow in nude mice, whereas they underwent neovascularization and became clinically evident when primary neoplasm was removed. In the absence of angiogenesis, micro metastases rarely exceeded 0.2-mm diameter and contained many proliferating tumour cells balanced by many apoptotic cells [27]. When they were allowed to become angiogenic, they grew rapidly. Dormancy may be generalizable to a variety of tumours in which blocked angiogenesis, resulted in balanced tumour cell proliferation and apoptosis [28].

2.2. Role of angiogenesis in diabetic retinopathy

Ischemia and hypoxia are conditions at which the retina uses a common response pathway which is employed by most, if not all, tissues to activate specific genes to deal with the crisis and restore oxygen homeostasis, for example, by restructuring blood supply [29]. Deregulation of this restoration pathway can lead to retinal neovascularization. Several lines of evidence indicate that the progression of pathologic and physiologic angiogenesis is tightly controlled by a balance of pro-angiogenic and anti-angiogenic stimuli. Angiogenesis in retinopathy occurs through a progression of events starting with the activation of endothelial cells located within normal vessels nascent to the source of stimulation [30,31]. In most cases, this activation occurs through the action of angiogenic cytokines but may also be initiated through various other means. Ultimately, the new capillary formation is carried out through a series of steps, which may be broadly categorized into an early and late phase. Events in the early phase tend to function in creating the new endothelial cell tubes followed by events in the late phase resulting in the stabilization and spatial orientation of the new micro vessels developed. The increased levels of blood glucose at hyperglycemic conditions is considered the most critical factor in regulating retinal blood flow, due to its structural and physiological effect on retinal capillaries making them functionally and anatomically incompetent. The efficacy of development of proliferation, tubules formation and vascular permeation mediating angiogenesis in retinopathy may depend on the imbalance between the growth factors (leading to angiogenesis) and angiogenic inhibitors. Other than angiogenesis mediated proliferation, the imbalance between the ROS generated and the antioxidant defense system also leads to development of diabetic retinopathy which is due to increased level of oxidative stress [32–37]. These evidences states the effective role of angiogenesis in developing ocular diseases and thereby makes clear that, preventing angiogenesis leads to inhibition of sustained development of ocular diseases.

2.3. VEGF in retinal angiogenesis

Several growth factors are elevated during the angiogenesis in retinopathy followed by the abnormalities in their respective metabolic pathways and among which VEGF has a distinctive role in the elevation. Thus here we mainly focus on the role of VEGF in angiogenesis development and their pathogenesis [33–35].

Vascular endothelial growth factor [VEGF] is a potent, endothelial cell mitogen that stimulates proliferation, migration and tube formation leading to angiogenic growth of new blood vessels [38]. VEGF (or VEGF-A) is a heparin-binding homodimeric glycoprotein of 45 kDa, belonging to a gene family which includes VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PLGF). As the prototype member of this gene family, VEGF is a key regulator of blood vessel growth. VEGF-C and VEGF-D regulate lymphatic angiogenesis. It exists in at least six isoforms (121-, 145-, 165-, 183-, 189-, and 206-amino acid isoforms) in human and three isoforms (120-, 164-, 188-amino acid isoforms) in the rodent. These isoforms are generated via alternative splicing from a single VEGF gene. $VEGF_{121/120}$ is freely diffusible whereas $VEGF_{189/188}$ and $VEGF_{206}$ are primarily associated with the cell surface and the extracellular matrix (ECM). $VEGF_{165/164}$ has both properties, as a fraction is diffusible while some is sequestered. Different VEGF isoforms possess distinct functions in vascular development among which $VEGF_{165/164}$ may plays an important role in pathogenesis of retinal revascularization. Studies of clinical specimens have shown a strong correlation between increases in intraocular VEGF levels and the development of PDR [38–41]. Several studies in animals and humans have proven the role of VEGF in retinopathy. It was Michaelson who first suggested that it was an angiogenic factor that was responsible for neovascularization in retinopathy [42], but it was not until the early 1980s that VEGF was identified as the key angiogenic molecule [43,44]. VEGF also is thought to have a key role in the pathogenesis of diabetic macular edema (DME) due to its action in increasing vascular permeability. Studies in diabetic animal models have shown that retinal vascular alterations resembling background diabetic retinopathy are associated with increases in retinal VEGF levels and increased expression of VEGFR2 [35,36]. Furthermore, blocking VEGF function can prevent diabetes induced permeability increases in both patients [45–48] and animal models [49–51], implying a direct role for VEGF in this pathology. VEGF levels are increased in ocular fluid from patients with diabetic retinopathy [52,53] and other retinal neovascularizing diseases. In situ hybridization analyses showed the proliferation of blood vessels to be accompanied by an induction of retinal VEGF expression only in the retinal layer affected by decreased perfusion [54]. Also, transgenic mice with over expression of VEGF in the photoreceptor layer develop intraregional and sub retinal neovascularization [55]. Gene expression of VEGF which is a tightly regulated process has profound over expression leading to pathological consequences in a variety of disease conditions, including diabetic retinopathy [36,40]. In addition to hypoxia, a number of factors contribute to the over expression of VEGF in a diabetic retina, including TGF-b, TNF-a, IGF-1, advanced glycation end products and oxidative stress, all of which are increased during diabetes [56–60]. Thus, the biological activity of VEGF is tightly linked to its expression level that states the need for the balanced level of the growth factors.

2.3.1. Expression of VEGF receptors in retinopathy

VEGF receptors belong to the super family of tyrosine kinase receptors. Both receptors have seven immunoglobulin-like domains in the extracellular region, a single transmembrane domain, and a split intracellular tyrosine kinase domain. VEGFR-2 is the major mediator of biologically-relevant VEGF signaling in endothelial cells. In endothelial cells, the biological effects of VEGF are mediated through its high affinity receptors, VEGFR-1 (flt-1) and VEGFR-2 (KDR in human/flk-1 in the rodent).The major retinal cell types capable of producing and secreting VEGF, include the retinal pigmented epithelium (RPE) [37], astrocytes [61], Muller cells [62], vascular endothelium [63] and ganglion cells [64]. The responses to the hypoxia determine the efficacy of the cells among which Muller cells and astrocytes generally produce the greatest amounts of VEGF under hypoxic conditions [65]. In vitro mRNA degradation assays clearly states the evidence for increase in VEGF mRNA stability in response to hypoxia that have led to the identification of adenylate/uridylate-rich element (AREs) in the 30 UTR of VEGF mRNA. The half-life of VEGF mRNA increases by 2–3-fold in hypoxic conditions [66] due to the stabilizing effect of HuR, a 36 kDa RNA-binding protein, which binds with high affinity to AREs in the 30-UTR of VEGF mRNA, thereby avoiding their degradation by endonucleases [67,68]. Cultured retinal pericytes showed expression of VEGFR-1, rather than VEGFR-2, whereas cultured RPE cells expressed both receptors lead by oxidative stress. A significant increase in VEGFR-2 was found when intraocular inoculation with herpes virus was provided in ganglion cells of mouse [69]. VEGF expression is first seen in trophoblast cells within a few days of implantation, and expression is highest in the choroid plexus and ventricular epithelium in a mouse developing retinopathy. Studies in newborn mice using the VEGFR-specific kinase inhibitor,

SU5416, indicate that Muller cell survival or proliferation during retinal development is VEGFR- and MAPK-dependent, in which VEGFR-1 and -2 were found on uterine smooth muscle cells in vivo. In the human retina VEGFR-1 and -2 can be expressed by neural, glial and vascular elements. In a study of patients with diabetic retinopathy, VEGFR-1 expression dominated in normal retina, but was not increased in the diabetic retina, while VEGFR-2 levels were increased, particularly in the vascular elements [70]. VEGF is a relatively endothelial cells specific mitogen, responsible for promoting endothelial cell growth and survival [71–74]. VEGF also causes increased vessel permeability that requires activation of several receptors and growth factors, among which VEGF plays a vital role in proper physiological angiogenesis. Evidence for the interaction between VEGF, VEGF receptors, and neuroglial cells of the retina in normal vascular development is been proven from various studies. In addition to the mitogenic influence on endothelial cells, VEGF also functions as a survival factor. These survival effects are developmentally regulated, as inhibition of VEGF results in apoptotic changes only in the neonatal vasculature. The expression of VEGF in the developing retina has been examined in various species and in the human fetes. In both animals and humans, VEGF expression is initially seen in the astrocytes of the neuroblastic layer adjacent to the inner limiting membrane near the optic disc, and it advances towards the periphery with a gradual down regulation in the central retina. VEGF expression always precedes the advancing blood vessels until the vessels reach the peripheral margin of the retina. With advancing age, in rat and kitten retinas, VEGF expression disappears from the superficial layer, and there is a second wave of VEGF expression in the Muller cells of the inner nuclear layer. This correlates with the formation of the deep vascular plexus. A strong spatial correlation between VEGF expression and increased neuronal differentiation and metabolic activity was revealed in the human fetal retina from similar findings [70]. A collective report over the above studies demonstrates that VEGF expression in the retina is progressively and wholly related to vascular developments.

2.4. Other factors

VEGF being known for its major contribution towards the development of angiogenesis mediating retinopathy, there are several other factors involved in the development of complications in diabetic retinopathy. An analysis for the ocular angiogenic factors in the analysis of human tissue (retina/choroid, RPE cells and endothelial cells) and vitreous using ELISA, immunohistochemistry, in situ hybridization or zymography showed that the following factors namely PDGFa/b, aFGF, bFGF, CTGF, TGFb, HGF, IL-6, IL-1, TNFα, EGF, MCP-1, PLGF, IL-8, M-CSF, Angiopoietin-1, Semaphorin-3A Erythropoietin, Angiopoietin-2, Adrenomedullin, Leptin, SDF-1, IGF-1, MMP-2, MMP-7 and MMP-9 were responsible for angiogenesis development leading to complications such as AMD and diabetic retinopathy [75,76].

3. Drug developments in retinal angiogenesis — an overview

Technology advancements have made therapies for retinal diseases of neovasculature a potential field of research in developing countries. Various researches on anti-cancer, anti-angiogenic agents have fuelled the way for ocular therapeutics. The market size for age-related macular degeneration and diabetic retinopathy is huge. Big pharma and biotech are competing in developing drugs capable of having reliable effect on ocular neovascular diseases, on the research level, towards a serious activity to bring such technologies to the clinic. Finally, over the last three years, triple digit million dollar business development deals have been consummated, mostly for VEGF-A targeted modalities. Such bio dollar partnerships were the eye openers which have now led to a concerted action to develop ocular drugs to combat ocular neovascularization.

3.1. Recent approaches in development of drugs for retinopathy

The need for the development of the retinal drugs have dramatically increased due to their low availability and cost specificity. In the last 12 years only 24 drugs have been approved for ocular therapies in comparison to 61 drug approvals for cancer [70]. To date, no molecular therapies have received FDA approval for the treatment of diabetic macular edema (DMO), proliferative DR or ROP [77]. It is to be believed that, only three of these 24 agents were directly aimed at ocular neovascularization. Of those three technologies, two were developed as anti-angiogenic and interestingly both targeting the pro-angiogenic cytokine VEGF-A. The first technology to be approved in the year 2000 was Visudyne (verteporfin for injection) for the treatment of exudative age-related macular degeneration by QLT. This technology utilized laser ablation with a photosensitizing agent that aided in the destruction of pathological ocular vessels. The second approval came 4 years later, in 2004, with Macugen (pegaptanib for injection] for intravitreous use given every 6 weeks. It is a pegylated aptamer that binds preferentially to the heparin binding domain of the VEGF protein, and is marketed to selectively inhibit the "pathologic" VEGF165 isoform towards the treatment of exudative age-related macular degeneration. Macugen proceeded through clinical trials and gained the distinction of being the first "anti-angiogenic" drug to be approved by the FDA for ocular application. Currently Macugen is owned by Pfizer and OSI Pharmaceuticals. More recently, in June of 2006, Genentech received approval for Lucentis (Ranibizumab for injection], a Fab fragment of an antibody able to bind and neutralize VEGF-A, for the treatment of exudative age related macular degeneration [76,78]. As of January 2007, there were 98,722 federally and privately supported clinical trials registered with the FDA among which 1031 clinical trials (1%) designated for the eye as compared to 12,301 (12%) for cancer. Of these 1031 trials, 14% (145 studies) were aimed at age-related macular degeneration and 8% (82 studies) for diabetic retinopathy. There are at least 60 agents in the clinic touted as anti-angiogenic, with 2/3 having activity outside of VEGF-A inhibition. Many more potential anti-angiogenic candidates are currently in preclinical development, with the distinct possibility of moving into ocular clinical studies. Hence, the window of opportunity remains open for new drugs to synergize with the VEGFA antagonists now approved for ocular application. A data from Sherris Pharma Partners (Jan 2007) reveals that Avastin an Anti-VEGF mAb inhibitor have been approved for AMD and DR suggesting IVT. The other drugs under clinical trials are TG100801 owned by TargeGen which is a Src kinase inhibitor under Phase trial I, Ruboxistaurin owned by Lilly which is a PKC b inhibitor under phase trial III and Octreotide owned by Novartis Growth hormone, IGF-I peptide inhibitor under phase trial III [76].

Thus these meagre approvals for therapies for retinal angiogenesis state the urge for the need of therapeutic developments in the field of research for ocular angiogenic targets.

3.1.1. Agents targeting VEGF receptors

Currently there are three available anti-VEGF agents namely pegaptanib, bevacizumab, and ranibizumab. Several others are under preliminary considerations for phase trials. Pegaptanib (Macugen, OSI/Eyetech, and Melville, NY) is a pegylated aptamer directed against the VEGF-A 165 isoform. It was the first FDA-approved ophthalmologic anti-VEGF agent, for the treatment of choroidal neovascularization from age-related macular degeneration (AMD). In phase 2 it showed a profound effect in the improvement of anatomic and visual outcomes in patients with DME. Phase 3 trials of pegaptanib for DME are currently being conducted. Bevacizumab (Avastin, Genentech, Inc., South San Francisco, Calif), a full-length

recombinant humanized antibody, is active against all isoforms of VEGF-A. It is FDA-approved as an adjunctive systemic treatment for metastatic colorectal cancer. The agent has shown greater promise through its intravitreal medication. Plans for a phase 3 trial of two doses of an intravitreal anti-VEGF agent versus modified ETDRS grid laser photocoagulation for DME are under discussion. Ranibizumab (Lucentis, Genentech, Inc., South San Francisco, Calif), a recombinant humanized antibody fragment, is active against all isoforms of VEGF-A. Intravitreal ranibizumab is FDA-approved for the treatment of exudative AMD. The risk of injection-related endophthalmitis with the anti-VEGF agents is variable, but appears to be lower in more recent studies. VEGF Trap is a molecule that contains immunoglobulin domains of both VEGFR-1 and VEGFR-2 fused to the constant region of human IgG. It functions as a high-affinity soluble receptor that binds and neutralizes both VEGF and placental growth factor. Systemic VEGF Trap reduced CNV volume determined by OCT in a trial of 25 patients. Still, the side effects seen from these clinical trials provide further evidence of the importance of VEGF in normal adult homeostasis. It is interesting to note that the mechanism for preeclampsia, a condition arising during pregnancy and associated with similar systemic effects as those resulting from systemic bevacizumab (proteinuria, edema, and hypertension), is believed to be promoted by increased levels of circulating soluble VEGFR-1 (s-Flt-1), which sequesters VEGF and prevents it from downstream signalling. Also, similar side effects were reported in a study testing another anti-VEGF agent when administered systemically, VEGF Trap (Regeneron) [76].

3.1.2. Laser therapy

The only available laser treatment for advanced PDR is the laser photocoagulation in which the result is presuming in case dependence. It is carried out by destroying retinal tissue which diminishes retinal hypoxia and VEGF expression by lowering down the metabolic demand. The therapy was effective in most cases but some undesired or fatal effects such as the decreased peripheral vision, impaired night vision and change colour perception was confound due to loss of retinal tissues. Moreover, the retinopathy will sometimes progress despite appropriate treatment. Therefore, there is a great need for the development of new therapies to prevent and treat diabetic retinopathy [78,79].

3.1.3. Future prospects of the retinal drugs

The global prevalence of AMD (age-related macular degeneration) and diabetic retinopathy are increasing due to rising numbers of older people worldwide. In addition, the world is facing a “diabetes epidemic”, with rapidly-increasing numbers of people with diabetes and diabetic retinopathy proving a clear market opportunity existing for pharmacological treatments for diabetic retinopathy, dry AMD and other retinal disorders. Clinical experience with pharmacologic treatment for diabetic retinopathy continues to increase and reported outcomes in observational case series are promising [80,81]. At this time, improved metabolic control and local ocular treatments (photocoagulation and vitrectomy) remain the proven. From 2009 to 2024, such treatments could repeat the commercial success that Lucentis achieved in the treatment of wet AMD. The latest pharma report — World AMD and Diabetic Retinopathy Pharmaceutical Market 2009–2024 — examines prospects for the retinal disorder market over the next 15 years. Although none of the pharmacologic agents discussed have crossed over phase trial III for treatment of patients with diabetic retinopathy, off-label treatment can be considered for patients unresponsive to traditional standard care. In patients with complications of PDR not amenable to photocoagulation, intravitreal anti-VEGF agents may produce short-term stabilization or regression of iris and/or retinal neovascularization [76].

The above information suggest that, ocular drugs that are approved and are under clinical trials give the impression to be mainly VEGF targeting and the interference with their downstream pathways responsible for angiogenesis. VEGF blocking therapies appears very promising in the treatment of cancer, since they have been directed only against migrating and proliferating capillary endothelial cells at a site of angiogenesis [82]. Hence there is a surplus need for the development of drugs capable of eradicating neovascularization targeting and inhibiting the pathways leading to angiogenesis, with their known underlying mechanism.

3.2. Challenges and issues of drug development for retinopathy

There is no any clear approach for the elimination of the initial stages of diseases of both retinal and sub-retinal origin i.e. diabetic retinopathy and age-related macular degeneration though they are being chronic. Neovascularization may utilize multiple angiogenic pathways, to which an ideal anti-angiogenic agent may inhibit and stabilize the disease but also expected to improve the vision. Furthermore, late stage disease creates problems outside of neovascularization (i.e., retinal scarring and detachment). In order to evaluate drugs for retinal diseases characterized by ocular neovascularization, several criteria's such as (i) they must be capable of preventing or inhibiting disease progression caused by the growth of pathological vessels i.e. a ‘magic bullet’ (ii) targeted delivery without affecting the native cells thereby inhibiting vision loss (iii) less/non toxic (iv) the binding of the drugs to non-specific receptors to be avoided (iv) be formulated for long term drug delivery and disposal of the unreacted drugs or by products, should be satisfied by the drug. Agents able to cause non-specific but reversible damage to existing vessels i.e. Vascular Disrupting Agents (VDAs) are also considered the important characteristics of the drug developed against angiogenesis but toxicity is a major factor unsatisfied when treated to normal vessels. VDAs are believed to exert activity by reducing blood flow to vessels. How these agents would work in the eye is still not entirely clear. Unfortunately, as these agents do affect normal vessels, and they may possess toxic consequences especially if there is partial non-pathological blockage of the normal vessel. Evidence of this event has been shown in cancer patients where these agents have caused cardiac compromise leading to myocardial infarction Furthermore; systemic exposure of VDAs could make systemic toxicity a possibility. Thus a drug developed against neovascularization is modulated on endothelial cell function (e.g. proliferation and migration) or inducing apoptosis in endothelial cells to inhibit pathological angiogenesis. Among many pro-angiogenic cytokines capable of inducing angiogenesis being identified, clinical researches are now focussed on developing “designer” molecules i.e. small molecule tyrosine kinase inhibitors activating endothelial cell surface tyrosine kinase receptors. In addition, biologic agents targeting the expression and/or function (e.g., binding, neutralization) of pro-angiogenic cytokines have been popular choices of drug development companies.

Hence developing biological agents with broad anti-angiogenic or vascular activity that can inhibit the late stage effects of retinal detachment provide long-term effects and have a good safety profile to the current requirements in ocular therapies.

4. Ophthalmological therapies aches economic advancements

In 1990 there were only three classes of drugs for diabetes treatment namely insulin (animal or human), sulfonylurea and metformin but nine classes are now available. The newer agents have expanded the range of clinical choice with individual benefit to many patients, but they have also greatly increased the cost of treatment. Approximately 16 million Americans have diabetes, with 50% of them not even aware that they have it. Of these, only one half receives appropriate eye care. Thus, it is not surprising that diabetic retinopathy is the leading cause of new blindness in persons aged 25–74 years in the United States. Approximately 8000 eyes become blind

yearly because of diabetes. The treatment of diabetic retinopathy entails tremendous costs, but it has been estimated that this represents only one eighth of the costs of social security payments for vision loss. This cost does not compare to the cost in terms of loss of productivity and quality of life. This is because, though there have been so many drugs developed for their VEGF targeting capability and anti-angiogenic property there is a major problem with the economic condition for the various production costs for the development and marketing of these drugs [76]. Due to the high significant characteristics of the drug to be confirmed, various cost-specific analysis and measurements have to be carried out which makes development of these drugs a cost-expansive one. Thus an alternative or a remedy for ocular therapies is the need of the era that should provide a cost effective treatment reaching every people of even lower economic status.

5. Silver nanotechnology invades therapies for angiogenic retinal disorders

Silver has been a metal that came into use even before Neolithic revolution. Even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during 8th century [83]. Silver powder was believed by Hippocrates, the father of modern medicine, to have beneficial healing properties and listed as a treatment for ulcers. Earlier, in the 19th century, microbial infections were treated with 0.5% $AgNO_3$ like ophthalmic neonatorum (by German obstetrician Carl Creed), and for the prevention of infection in burns. Prior to the introduction of the sulphadiazine cream, dilute solutions of silver nitrate were used to treat infections in the 19th century [84]. Silver-based antimicrobials can be effective in the treatment of infections on account the non-toxicity of active Ag^+ to human cells [85]. However, the use of ionic silver has one major drawback; they are easily inactivated by complexation and precipitation thus limiting the uses. Here zerovalent silver nanoparticles can be a valuable alternative for ionic silver. When the era of the antibiotics began with the discovery of penicillin, the use of silver slowly diminished. But in the present scenario due to the emergence of biocide-resistant strains, once again the use of silver for treating infections has gained importance. There are many methods available for the synthesis of silver nanoparticles [86]. Most chemical methods use a reducing agent (e.g., sodium borohydride) to reduce Ag^+ to Ag0 and a stabilizer (e.g., polyvinylpyrrolidone) to control particle growth and prevent aggregation. However, these chemical fabrication methods often have problems with particle stability and are difficult to scale up. In addition, there is a demand for more environment-friendly production methods. Alternatively, silver nanoparticles can also be synthesized biologically using microbes such as *Bacillus subtilis* and *B. licheniformis* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria). The distinctive property of silver capable of synthesizing nanoparticles at a specific concentration (1 mM) and inducing apoptosis in another concentration (5 mM) makes it highly preferable in the size dependent therapeutic applications [87–89]. Silver was known only as a metal till the recent past and it is when the nano era came into existence, people started to believe that silver could even be produced at the nanoscale. The recent emergence of nanotechnology has provided a new therapeutic modality in silver

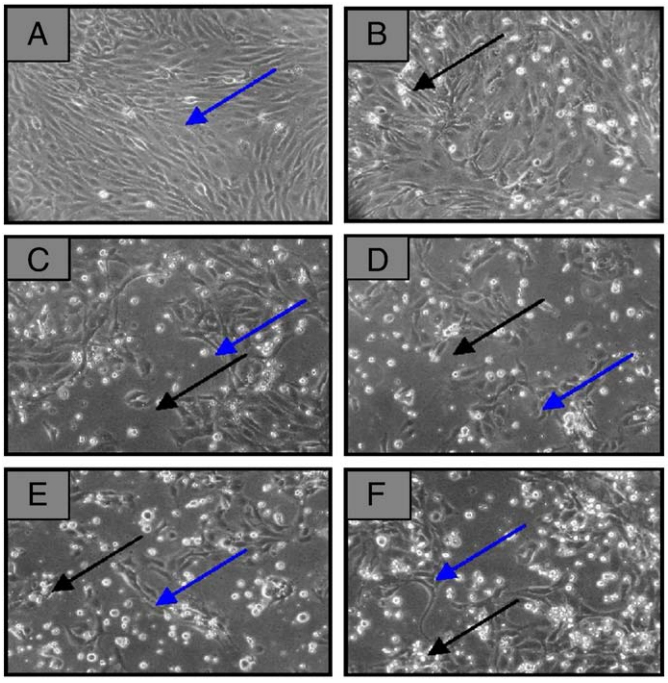

Fig. 1. Silver nanoparticles induce cellular morphology. Silver nanoparticles induce morphological changes in BRECs. BRECs were treated with (A) 10% FBS (B) 100 nM AgNPs (C) 200 nM AgNPs (D) 300 nM AgNPs (E) 400 nM Ag NPs (F) 500 nM AgNPs and were photographed under phase contrast microscope. The normal cells seemed to be cobble shaped and the dead cells are of spherical shaped, which previously showed the morphology of apoptotic bodies. (Blue arrow shows normal cells and black arrow indicates dead cells) [98].

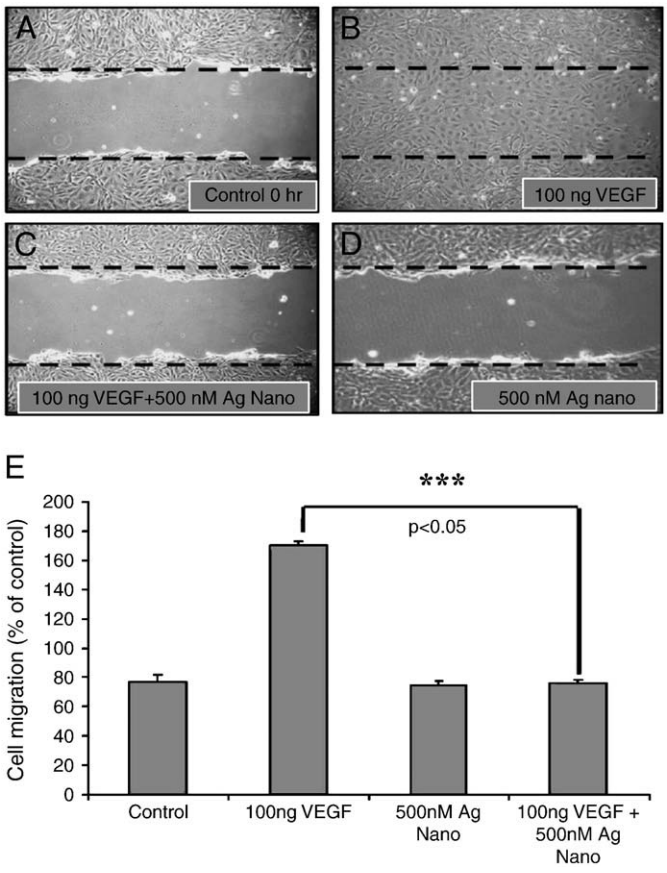

Fig. 2. Silver nanoparticles inhibit VEGF induced endothelial cell migration. Confluent monolayer of BRECs with various treatments was wounded and wound closure was monitored after 24 h and photographed using digital camera. Control cultures at 0 h (A). When VEGF is added, more endothelial cells migrated from the margin (B) when compared with the control (B) and the wounded area was closed by 24 h. While significant area of wound is uncovered in plates treated with 500 nM of silver nanoparticles in the presence (C) and absence (D) of VEGF by 24 h and the similar phenomenon was observed in plates. Panel E shows the quantitative measurement of migration assay as described in methods. The asterisk (*) represented significantly different value from control, $p<0.05$, data were mean ± SD calculated from three individual experiments ($n=3$; $^*p<0.01$, $^{**}p<0.001$, $^{***}p<0.0001$) [98].

nanoparticles for use in medicine [90]. Silver nanoparticles are also reported to possess anti-fungal activity, anti-inflammatory effect, anti-viral activity, anti-biofilm activity and anti-bacterial activity. But, silver nanoparticles can be well applied in therapy safely when the effective concentrations of silver nanoparticles on various size and shapes are determined. The surface chemistry of the nanoparticles controls the cellular uptake [91]. The toxicology is an important factor that has to be taken under consideration in any therapeutic molecule. Silver nanoparticles are proved to be non-toxic at specific concentrations, at which their activity is also profound to be therapeutic [89].

Any drug developed through biological means may contribute preferences towards their significant properties such as cost-economic, efficacy, biocompatibility and non-toxicity. Silver nanotechnology is one of the most advanced developments in the field of nanomedicine that finds its wide application in electronics, forensics, medicine etc. It has also been proven for its vivid therapeutic properties against angiogenesis and vascular developments [92] that pave way for its effective use in treating various angiogenesis related retinal problems. This is similar to TZDs and telmisartan that possess demonstrable anti-proliferative, and anti-inflammatory effects, *in vivo*, were shown to ameliorate PDR and CNV in rodent models, implying their potential efficacy for treating proliferative retinopathies in humans [93] but the preference for AgNPs lies on the fact that they are cost economic, feasible and biocompatible. Though the Anti-VEGF-A technologies dominate the field of development of drugs towards ocular therapies, agents with broader activity that occurs later down the angiogenic pathway and those drugs which are capable to synergize with anti-VEGFA technologies will dominate the next wave in ocular diseases of neovascularization [94]. Aromatic compounds, such as tyrosine kinase inhibitors, are capable of penetrating the blood-retinal barrier because of their small lipophilic structure enabling transcellular migration. Systemic delivery of such treatments has been tried. But the technology has some systemic drawbacks due to side effects [95] In this scenario silver nanoparticles being a inhibitor of Src tyrosine kinase is capable of targeting and inhibiting the downstream pathways of ocular angiogenesis and proves itself as a cost effective therapeutic remedy to ocular treatments (Fig. 1). In spite of the therapeutic applications, the clear studies over the internalization process and the final intracellular location of the silver nanoparticles within the retinal cells would assure the role of these nanoparticles for effective therapies.

6. Silver nanoparticles — treating proliferative retinopathy

Angiogenesis is a complex process, involving multiple gene products expressed by different cell types, all contributing to an integrated sequence of events [96]. Ocular angiogenesis cascades the activation of endothelial cells located within normal vessels nascent to the source of stimulation, leading to migrations, vascular permeation and tubule formations that causes several retinal complications such as neovascularization. In elderly patients with diabetic retinopathy or age-related macular degeneration, normal ocular vessels may be affected by VDAs and result in hypoxia, ocular cell death, and subsequent neovascularization [94]. In the cases stated above in this context the silver nanoparticles have been proven for their applicable properties in treatment for retinopathy that have been clear from the various research carried out by our team.

6.1. Silver nano — an anti angiogenic molecule

The central role of VEGF in angiogenesis development serving as a primary target for the anti-angiogenic molecules capable of targeting the growth factor mediated angiogenesis for curing retinopathy is clearly understood from the discussions made above. Clinical trials have confirmed the importance of VEGF in the disease pathogenesis and the drugs developed against the downstream pathways (Fig. 2). In recent years the impending emergence of silver nanoparticles as a potent anti-angiogenic agent is made clear from several researches

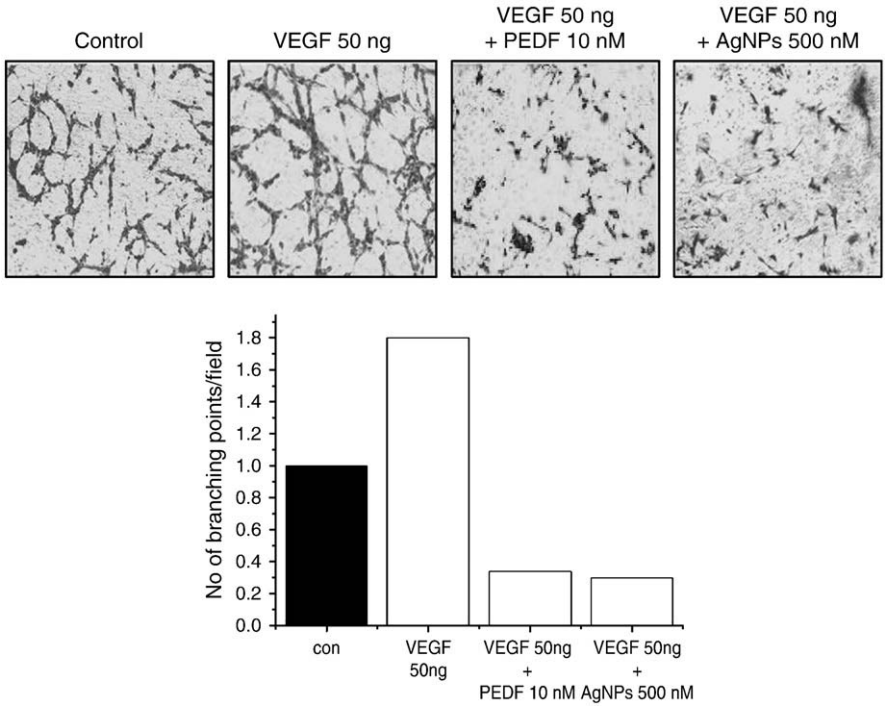

Fig. 3. Ag-NPs and PEDF inhibit VEGF-induced tube formation of endothelial cells. BRECs (1×10^5 cells) were inoculated on the surface of the Matrigel, and treated with VEGF (50 ng/ml) in the presence or absence of either 500 nM of Ag-NPs or 10 nM of PEDF. The morphological changes of the cells and tubes formed were observed under a microscope and photographed at 200× magnification. Tube formation was quantified by counting the number of connected cells in randomly selected fields at 200× magnification (Carl Zeiss, Chester, VA, USA), and dividing that number by the total number of cells in the field. Column shows the quantitative measurement of tube length. These experiments were performed thrice with similar results and significant differences from control group were observed ($p<0.05$) [92].

made [92,98]. Ag-NPs and PEDF inhibited the proliferation of ECs in similar trend, indicating the anti-angiogenic effect of Ag-NPs which is due to its direct effect on ECs. The effect of silver nanoparticles was observed in cell morphology itself. Silver nanoparticles-treated endothelial cells showed normotonic cell shrinkage, typical of apoptotic bodies (Fig. 3).

Consistent with the observations the data demonstrated an inhibitory effect of silver nanoparticles on VEGF induced proliferation, migration and tube formation in ECs (Figs. 4 and 5).

Silver nanoparticles were found to abrogate VEGF induced angiogenesis in bovine retinal endothelial cells and *in vivo* angiogenesis using mice and rat model (Figs. 6 and 7).

Since endothelium is the major target for many therapies, the effect of biologically-synthesized silver nanoparticles on VEGF induced angiogenesis in BRECs has been investigated. Studies report the biological functions of Ag-NPs as an inhibitor of angiogenesis by illustrating the inhibition of PI3/Akt signalling pathways and also inhibitory activity of angiogenesis *in vivo* (Figs. 8 and 9) [97].

The expression patterns of VEGF, a potent pro-angiogenic factor, and PEDF have been well-characterized in the eye, and the balance of their opposing stimuli prevented the development of neovascularization, which is involved in the development of PDR [94]. The research focused on the inhibitory effects of Ag-NPs on endothelial cell viability, cell proliferation, migration, and capillary tube formation in response to VEGF in BRECs. PI3K/Akt signalling plays a critical role in executing multiple cellular metabolic pathways and in particular cellular energy metabolism. It can mediate cell survival, cell proliferation, cell growth, and differentiation whereas down regulation of Akt activity leads to degenerative diseases. Akt is one of the key signalling molecules in cell survival and angiogenesis in the pro-survival function of VEGF. VEGF was able to activate Akt through phosphorylation and silver nanoparticles were able to block it. Furthermore, it also showed that Ag-NPs inhibit *in vivo* angiogenesis in comparable level to PEDF. In addition, silver nanoparticles and PEDF strongly inhibited VEGF induced capillary tube formation. Thus the study revealed that Ag-NPs are potent anti-angiogenic molecule that inhibits VEGF induced angiogenesis in BRECs through the inhibition of the PI3K/Akt cell-survival signal in a similar pattern of PEDF [98]. It further confirms that silver nanoparticles block the cell survival through inactivation of Akt. To explore the potential effect of inactivation of Akt on mitochondrial apoptotic pathway, the activity of caspase-3 was examined. The caspase-3 assay showed an increase in caspase-3 level during treatment with silver nanoparticles. Increase in the caspase-3 activity is observed during most of the apoptosis and silver nanoparticles are known to induce caspase-3 activity in cancer cell lines. In silver nanoparticles-treated cells DNA laddering was also observed. Therefore it can be clearly stated that silver nanoparticles induce apoptosis in BRECs, via blocking Akt phosphorylation and activating caspase-3 molecules [92,98].

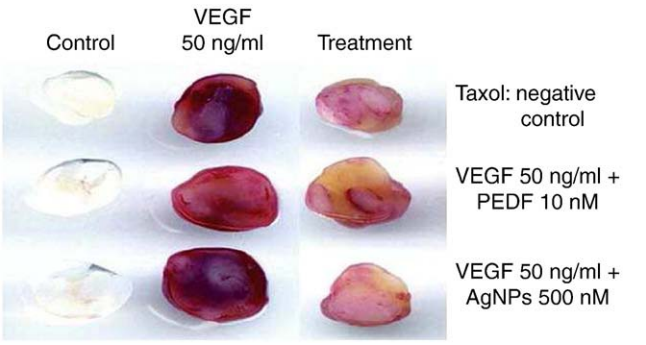

Fig. 4. Anti-angiogenic activity of Ag-NPs or PEDF using in vivo Matrigel plug assay Five- to 6-week-old C57BL/6 mice were subcutaneously injected with Matrigel containing Ag-NPs (500 nM) or PEDF (10 nM) with VEGF or without VEGF. After 7 days mice were sacrificed and representative Matrigel plugs were removed and photographed. Significant differences from control group were observed ($p<0.05$) [92].

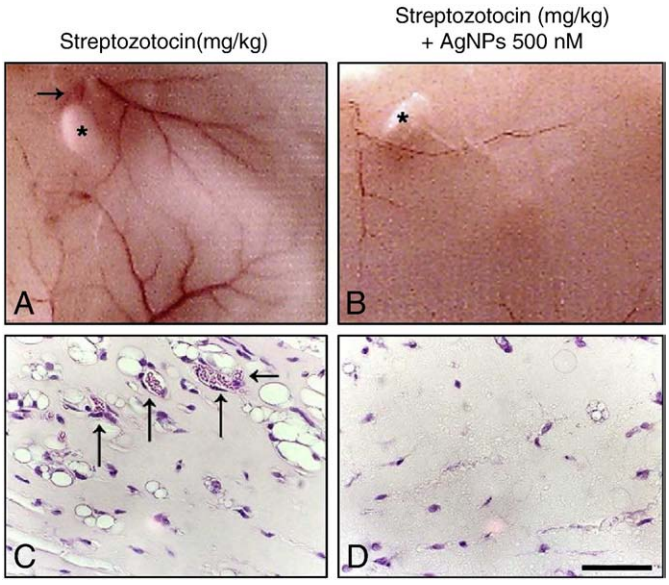

Fig. 5. Anti-angiogenic activity of AgNPs in vivo rat model. After rats were sacrificed, tissues were taken pictures. Top panel: Gross photographs of Day 7 Matrigel implants with skin vessel background. Representative figures show (A) Streptozotocin without Ag-NPs, (B) Streptozotocin plus Ag-NPs. Bottom panel: Histologic sections and hematoxylin and eosin stained cross-sections showing representative photographs obtained from the sections of retina stained by hematoxylin and eosin in rats (C, D). Significant differences from control group were observed ($p<0.05$) [94].

6.2. Silver nano: an anti-permeability agent

Vascular endothelial barrier dysfunction characterizes a diverse array of disease processes and plays an important pathophysiological role in many diseases including diabetic retinopathy. The development of new therapeutic strategies aimed at reducing excessive vasopermeability could therefore have serious clinical implications. In particular, the characterization of new molecules with anti-permeability properties and elucidation of their mechanisms of action could facilitate efficient treatments [101–106].

In our study, to determine the effects of silver nanoparticles (Ag-NP) on vascular endothelial growth factor (VEGF) and interleukin-1 beta (IL-1β) induced vascular permeability, and to detect the underlying signalling mechanisms involved in endothelial cells, Porcine retinal endothelial cells (PRECs) were exposed to VEGF, IL-1β and Ag-NP at different combinations and the endothelial cell permeability was analyzed by measuring the flux of RITC-dextran across the PRECs monolayer. It was found that VEGF and IL-1β increase flux of dextran across a PRECs monolayer, and Ag-NP block the solute flux induced by both VEGF and IL-1β. To explore the signalling pathway involved VEGF- and IL-1β-induced endothelial alteration, PRECs were treated with Src inhibitor PP2 prior to VEGF and IL-1β treatment, and the effects were recorded. Further, to clarify the possible involvement of the Src pathways in endothelial cell permeability, plasmid encoding dominant negative(DN) and constitutively active(CA) form of Src kinases were transfected into PRECs, 24 h prior to VEGF and IL-1β exposure and the effects were recorded. Over expression of DN Src blocked both VEGF-and IL-1β-induced permeability, while over expression of CA Src rescues the inhibitory action of Ag-NP in the presence or absence of VEGF and IL-1β (Fig. 10) [99,100].

Further, an *in vitro* kinase assay was performed to identify the presence of the Src phosphorylation at Y419. Thus the study states that VEGF and IL-1β stimulates endothelial permeability via Src

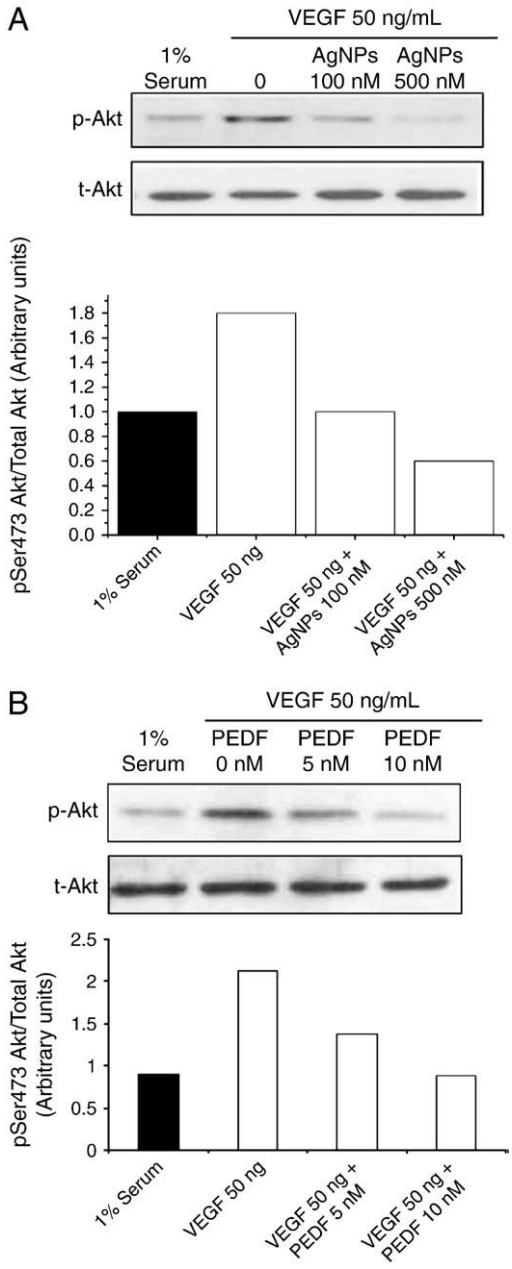

Fig. 6. Ag-NPs or PEDF inhibit VEGF-induced phosphorylation of Akt. A) Serum-starved BRECs were treated with VEGF (50 ng/ml) in the presence or absence of Ag-NPs 100 nM and 500 nM for 30 min. Equal amounts of total cell lysates were analyzed by Western blot analysis for phospho-Akt and Akt. Densitometric normalization of phosphorylated pAkt against Akt. Band density is shown in columns. All Western blot analyses were performed triplicates. B) Serum-starved BRECs were treated with VEGF (50 ng/ml) in the presence or absence of PEDF 10 nM and 5 nM for 30 min. Equal amounts of total cell lysates were analyzed by Western blot analysis for phospho-Akt and Akt. Densitometric normalization of phosphorylated pAkt against Akt. Band density is shown in columns. These experiments were performed thrice with similar results and significant differences from control group were observed ($p<0.05$) [92].

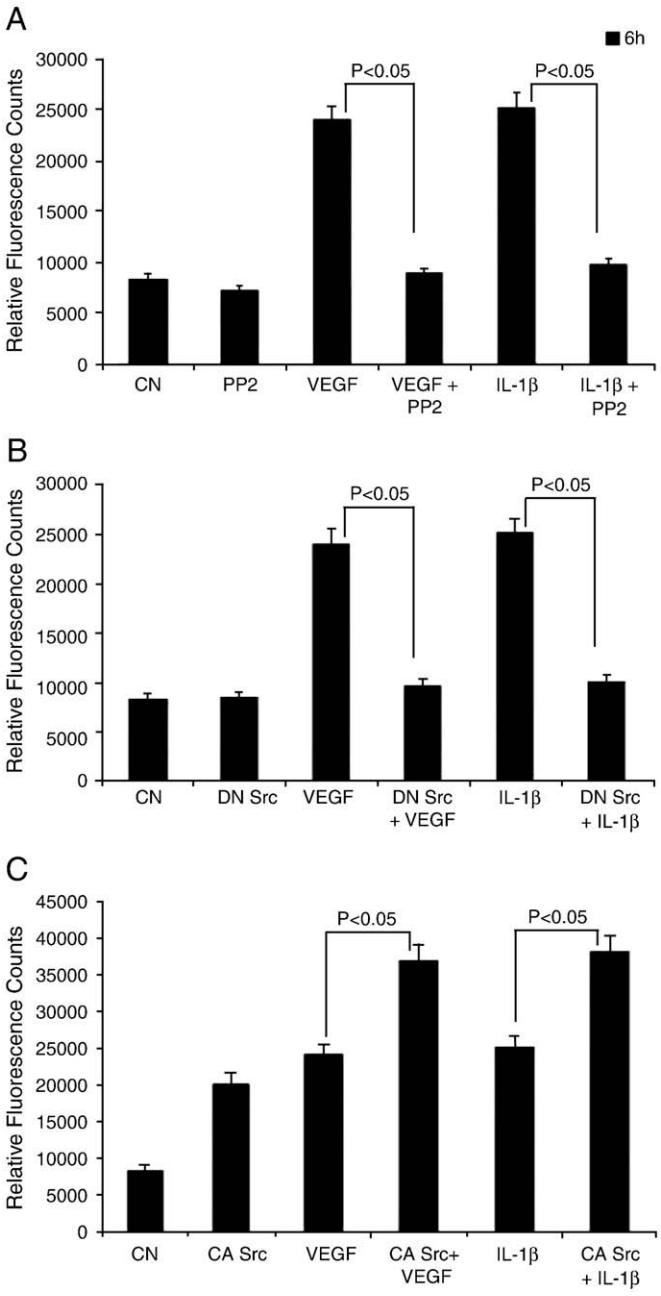

Fig. 7. Role of Src kinase activity in VEGF- and IL-1-induced endothelial cell permeability. (A) Effect of Src inhibitor on VEGF- and IL-1β-induced endothelial cell permeability. PRECs were grown to confluent monolayers on porous membranes (12-well transwell insert plate). The lower chamber was incubated with VEGF (25 ng/ml) or IL-1β (10 ng/ml) and in the presence or absence of PP2 (10 μM) for 6 h at 37 °C; (B–C) PRECs were transiently transfected with DNA dominant negative Src (HA-Src KD K295 M) and constitutive active Src (HA-Src-CA Y527F). Transfected PRECs were treated with VEGF and IL-1β for 6 h at 37 °C where the induction of permeability by growth factor in wild type cells was completely blocked in DN Src transfected cells (B) where the CA Src transfected cells resulted in increased permeability than the wild type cells (C). The flux of RITC-dextran from the upper to the lower chamber was measured 6 h after treatment. Values are expressed in relative fluorescence counts (RFCs) as mean ± SEM, with each condition performed at least in triplicate. [99].

dependent pathway by increasing the Src phosphorylation and Ag-NPs blocked the Src phosphorylation at Y419 [99].

The specific location of the nanoparticles during the treatment has been checked through various time intervals under a transmission electron microscope, which revealed that the nanoparticles of size ~50 nm were internalized during the treatment (Fig. 11).

In another study based on the determination of the effects of silver nanoparticles (Ag-NPs) on advanced glycation end-products (AGEs)-induced endothelial cell permeability revealed the anti-permeability nature of AgNPs [100]. Cultured porcine retinal endothelial cells (PRECs) were exposed to AGE-modified bovine serum albumin (AGEBSA) and the endothelial cell permeability was detected by measuring the flux of RITC-dextran across the PREC monolayers. Results revealed that AGE-BSA increased the dextran flux across a PREC monolayer and Ag-NPs blocked the solute flux induced by AGE-BSA. In order to understand the underlying signalling mechanism of

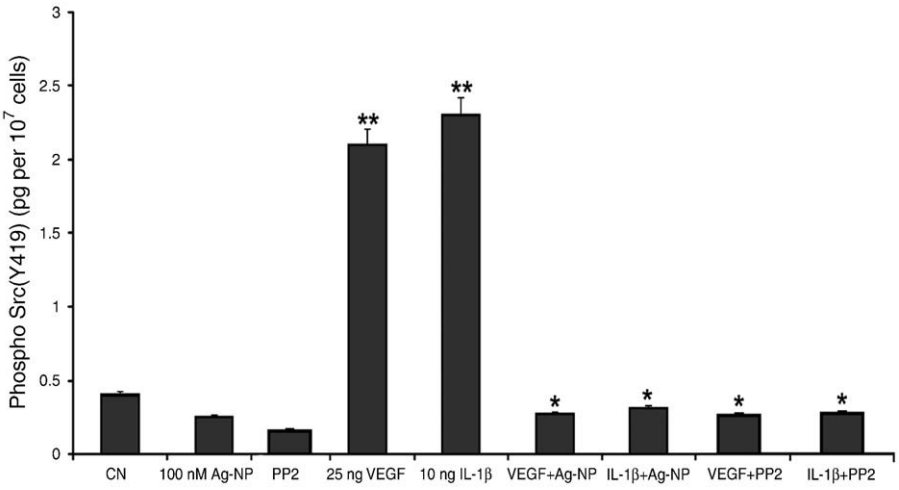

Fig. 8. Effect of Ag-NP and PP2 on VEGF- and IL-1β-induced Src phosphorylation [99].

Ag-NPs on the inhibitory effect of AGE-BSA-induced permeability, it is demonstrated that Ag-NPs could inhibit the AGE-BSA-induced permeability via Src kinase pathway. AGE-BSA also increased the PREC permeability by stimulating the expression of intracellular adhesion molecule-1 (ICAM-1) and decreased the expression of occludin and ZO-1 (Figs. 12 and 13).

Further, Ag-NPs inhibited the AGE-BSA-induced permeability by increased expression of tight junction proteins occludin and ZO-1, co-incident with an increase in barrier properties of endothelial monolayer. Together, the results indicate that Ag-NPs could possibly act as potent anti-permeability molecule by targeting the Src signalling pathway and tight junction proteins and it offers potential targets to inhibit the ocular related diseases. In developing new molecular therapies for vasopermeability disorders, it is important to recognize the spectrum of effects of the specific molecule which hereby confirms the anti-permeability property of AgNPs that makes them suitable for preventing retinal vascular hyper permeability and treatments against angiogenic targets.

7. Current technologies in targeted and controlled release of retinal drugs

The delivery of anti-angiogenic compounds to the choroid/retina without disrupting the integrity of the globe is highly desirable. Several researches on drugs developments for ophthalmological diseases are carried out in the decade and the major factors that every new drug developed relies upon is the targeting efficiency and the mechanism by which they act. The clear mechanism by which a drug is delivered and the mode of their action if known, can make the drug an efficient scope for therapeutic advances. Drug delivery to the retina is problematic and often resorting by invasive means such as repeated intraocular injections [107]. Depending on the particle charge, surface properties and relative hydrophobicity, nanoparticles can be designed to be successfully used in overcoming retinal barriers for targeted delivery systems [16]. The focus for an effective anti-angiogenic treatment relies on the major objective of sustained release with greater targeting efficiencies of a drug which can be

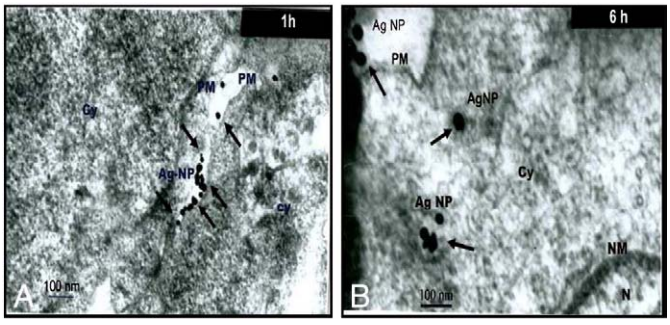

Fig. 9. TEM images of PRECs treated with silver nanoparticles. (A) Shows the image of the cells at 1st hour and (B) shows the image of the cells taken at the 6th hour. The latter image shows the silver nanoparticles internalized into PRECs (where PM — plasma membrane, Ag-NP — silver nanoparticles, Cy — cytoplasm, NM — nuclear membrane, N — nucleus) [99].

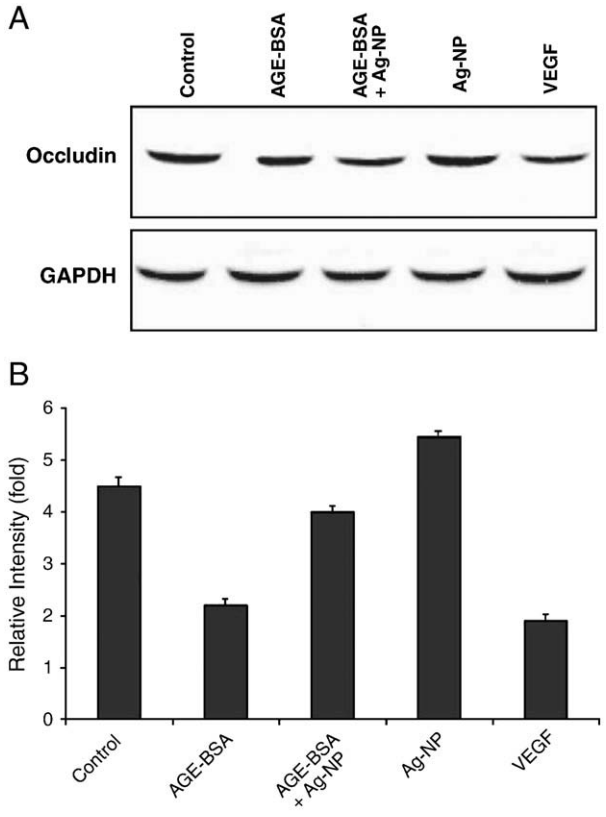

Fig. 10. Effect of Ag-NPs increasing occludin content in PRECs. (A cell lysates were immunoprecipitated with antibody to occludin. Precipitated proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and blotted with anti-occludin antibody; (B) Bar graphs represent averaged data quantified by densitometry of immunoblots, expressed as increase in fold. Data are representative of three independent experiments [100].

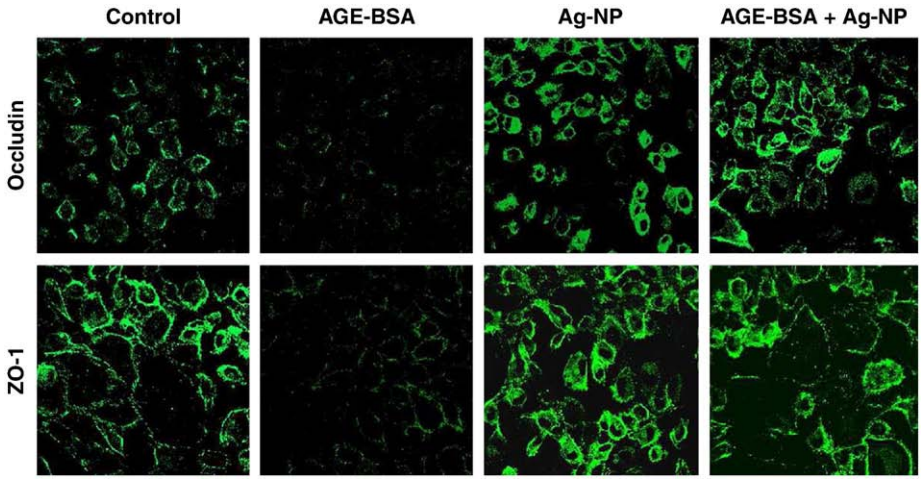

Fig. 11. Cellular distribution of occludin and ZO-1 PRECs monolayer cells [100].

accomplished based on RGD-conjugated polymer encapsulations carrying the drug i.e. silver nanoparticles.

7.1. Trends in targeted delivery using RGD peptides for retinal angiogenesis

Integrins are heterodimeric cell surface receptors found in early studies to mediate adhesion between cells and the extracellular matrix (ECM), by binding to ligands with an exposed arginine–glycine–aspartate (RGD) sequence. Integrin $\alpha_V\beta_3$ are over expressed in neocapillaries and in some tumor cells of various origins and are involved in tumor angiogenesis. These receptors also stimulate intracellular signaling through sphingomyelin pathway and gene expression responsible for cell growth, migration, and survival [108]. Targeting here can be carried out by the RGD peptides which are specific to the integrin receptors that are over expressed in the angiogenic and tumor cells alone [109]. The cyclic RGD peptide (c(RGDyK), cyclic arginine–glycine–aspartic acid–D-tyrosine–lysine) has been employed in targeted drug delivery system for malignant gliomas in the central nervous system [110]. The potential of cyclic RGD peptide is evidenced from various studies made over its targeting efficiency for tumor models. In fact, $\alpha_V\beta_3$ integrins have been demonstrated to be the participants in these diseases and can also be termed as disease markers for angiogenesis. Due to these significant properties, several researches have been focused on developing RGD peptides that could mimic cell adhesion proteins and bind to $\alpha_V\beta_3$ integrins. These RGD peptides are used widely as a targeting moiety for receptors because of their high affinity also [111]. Angiogenesis-related $\alpha_V\beta_3$-integrin expression is VEGF-rather than hFGF-dependent, and the efficacy of cyclic penta-peptid (RGDfV)-treatment in proliferative retinopathy is being proven effective as long as this integrin target is prominently expressed [112]. Surface-functionalized nanoparticles that have been conjugated using RGD peptides have proven for an effective targeted gene delivery to the neovascular eye on intravenous administration and potential to inhibit progression of laser-induced CNV in a rodent model [113]. The advantage of using the cyclic RGD peptides is on the fact that they posses their E residue side chain that are capable of binding to different kind of molecules such as drugs and fluorescent agent used for both targeted drug delivery and bioimaging.

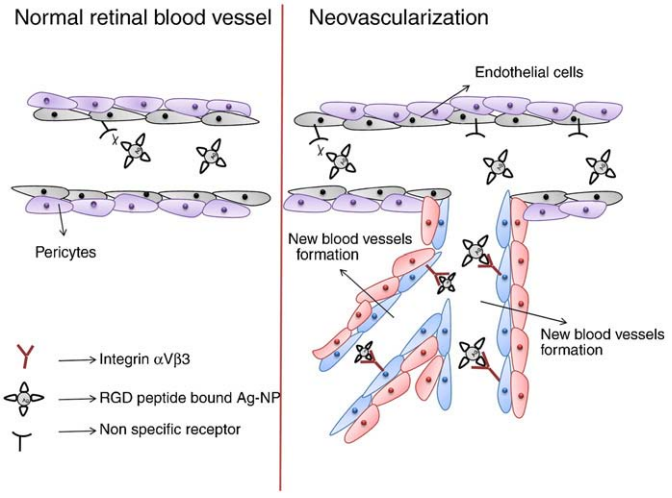

Fig. 12. The targeting mode of RGD peptide coated silver nanoparticles in the condition of neovascularization (new blood vessels — undesired) unaffecting the normal retinal cells. The RGD peptide does bind only to the over expressed integrin $\alpha_V\beta_3$ receptors that serve as a marker for the angiogenic cells. Thus the silver nanoparticles coated with RGD peptide evade the other non-specific membrane receptors in the normal cells, binding only to angiogenic cells.

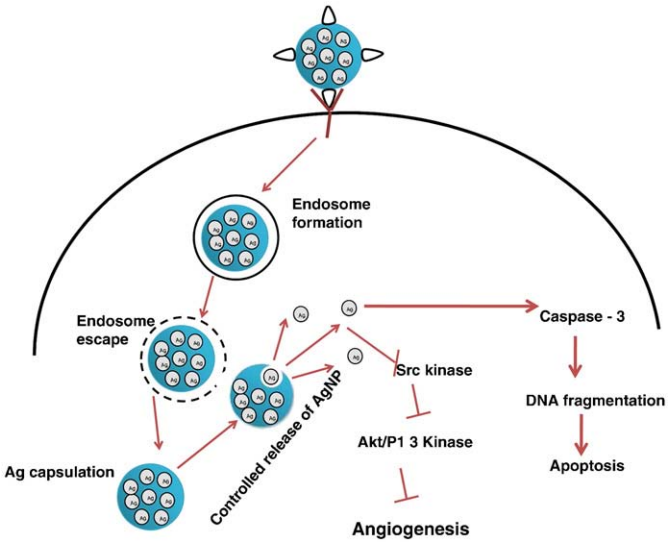

Fig. 13. The above figure details the proposed model for the mode of action by which the PGA capsulated silver nanoparticles conjugated with RGD peptide induce apoptosis in the targeted endothelial cells. The PGA encapsulated silver nanoparticles that target the specific integrin $\alpha_V\beta_3$ receptors are engulfed within the endosome through clathrin mediated activity and they are profound to endosomal escape. A controlled released of AgNPs is accomplished using the PGA and the released AgNPs inhibit the angiogenesis by restraining the Src kinase and Akt/P1-3 kinase activity within the cell. They also thereby augment the caspase-3 activity which leads to the apoptosis of the cells through fragmentation of the DNA.

7.2. Potential of PGA in controlled release of drugs

Controlled release of anti-angiogenic molecules for retinal therapies is a critical property of any drug being developed in ophthalmology. Polymer based delivery of drugs have greater advantage in carrying out this type of sustained release of the desired drugs. There are several evidences affirming the efficiency of polymer based drug delivery for enhancement of controlled release fighting ocular angiogenesis. Nanoparticles with CS dominated on the surfaces could efficiently open the tight junctions between Caco-2 cell monolayers as an efficient intestinal delivery system for peptide and protein drugs and other large hydrophilic molecules [114].

In a study made over the use nanogel composing of Ag nanoparticles bound to poly (N-isopropyl acrylamide-co-acrylic acid) as shell in bioimaging for cancer, the pH-responsive hybrid nanogel exhibited a sustained pH-controllable drug releasing behavior along with the high drug loading capacity [115] which thereby proved their efficiency of polymer coated drugs for lower drug requirements and controlled release during treatments. Among various polymers used in drug delivery like PEG [116], PLAs, poly-lactic acid (PLA) and copolymers with glycolic acid, making poly(lactic-co-glycolic acid) (PLGA) have an advantage of easily being biodegradable by breaking down into lactic and glycolic acids, both of which are metabolized via the Krebs cycle to carbon dioxide and water [117]. Triamcinolone loaded PLGA micro spheres (RETAAC) is being applied for treatment of refractory diffuse that has reached phase I/II trials for the DMO [118]. Poly-γ-glutamic acid (PGA) which is water soluble and biologically synthesized has also found greater potential resulting in controlled delivery and desired efficiency of the drugs. It is a hydrophilic, biodegradable, and naturally available biopolymer produced by a number of microbial species, most commonly, the *Bacillaceae* family. Its biological properties such as non-toxicity, biocompatibility, and non-immunogenicity qualify it as an important biomaterial in drug delivery applications [119]. In a recent study over the polymer based systems for drug delivery, nanosuspensions (200 nm) of the drug rilpivirine (TMC278) stabilized by polyethylene–polypro-pylene glycol (poloxamer 338) and PEGylated tocopheryl succinate ester (TPGS 1000) were studied in dogs and mice. A single-dose administration of the drug in nanosuspensions resulted in sustained release over 3 months in dogs and 3 weeks in mice, compared with a half-life of 38 h for free drug. This study evidences a proof-of-concept that nanoscale drug delivery may potentially lower dosing frequency and improve adherence [120] Addition of γ-PGA-NPs to the influenza HA vaccine revealed that γ-PGA-NPs enhanced the virus-specific protective immune responses [121]. γ-PGA has also known for greater preference in targeted delivery to solid tumors. Oral administration of the poly-gamma-glutamate is also known for inducing TLR4- and dendritic cells mediated anti-tumour effect [94] and also in another study supported for oral insulin delivery [122] These γ-PGA also possess multiple important roles in enhancing the cellular uptake and transfection efficiency of CS/DNA/g-PGA NPs making it highly applicable for gene delivery system [123]. An another study over the efficiency of γ-PGA coated nanoparticles in vivo showed an efficient control over the progression of the tumor with a greater increase in the controlled release of the drug along with an efficient targeting [124]. Thus in this review the application of PGA is preferred as a potent carrier for controlled release of silver nanoparticles in the targeted cells.

8. Model for targeted delivery and controlled release of silver nanoparticles for neovascularization

Research on nanoparticles-based drug delivery systems has gained tremendous interest worldwide, owing to their ability to deliver drugs to the desired target site for a prolonged period of time [77]. Silver nanoparticles here are preferred as a therapeutic alternative for anti-angiogenic therapies in the retina [97–100,125,126]. Their efficiency of targeting the neovascularized cells has to be deep-rooted in order to utilize them as a therapeutic remedy for retinal disorders. From the various evidences stating the potential of PGA in controlled release of drugs, along with the targeting efficiency of RGD peptides for the $\alpha_V\beta_3$ receptors that serve as biomarkers for neovascularized cells, a drug delivery system with amenable controlled release of the silver nanoparticles with respect to the time factor will enhance and ascertain their role in treatments for retinal angiogenesis.. Encapsulation of silver nanoparticles at a desired concentration preferably 500 nM will be optable with PGA capsulation in submicron level. The targeting of neovascularization cells can be accomplished by using a cyclic RGD peptide capable of binding its E residue side chain to the desired drug i.e. silver nanoparticles. Silver nanoparticles capsulated with Poly gamma glutamic acid may influence a double effect in control of angiogenesis with respect to the anti-tumor activity of Poly gamma glutamic acid. Thus here we propose a model for the targeted delivery of silver nanoparticles in the neovascularized cells along with a sustained control of release in the target cells thereby, avoiding the deterioration of the normal retinal cells. These same processes, if not properly regulated, can lead to thrombosis, inflammation, and cancer. From the various evidences stated above along with the proposed model we suggest that the targeting of the neovascularized cells using AgNPs can be carried out by developing silver nanoparticles bound to the cyclic E chain of the RGD peptide which is highly specific to the $\alpha_V\beta_3$ receptors that serve as biomarkers for neovascularized cells. The other major advantage is the luminescence property of silver nanoparticles at specific shapes and size that suspends the need for the use of photo thermal dyes during imaging. This proposed model of drug delivery using RGD peptides bound silver nanoparticles will serve as a remedy for ocular therapies especially in neovascularization (Figs. 12 and 13).

9. Conclusion

Angiogenesis an imperative phenomenon in the human system, through its various abnormalities and metabolic dysfunctions have led to an imbalance in the growth factors induced by changes in conditions within the system, thereby mediating proliferative retinopathy. Though there are several drugs been developed against ocular neovascularization, most strive the phase trials and they also are cost dependent. Nanomedicine is the use of nanotechnology to get pioneering therapeutic breakthroughs and have proven to be cost economic. The various findings and advancements in the field of silver nanotechnology have been able to address some of biggest questions regarding human life. Great strides are already being made in creating nanoparticles systems for targeted drug delivery. Silver nanoparticles are better than conventional ophthalmic drug forms to enhance bioavailability without blurring the vision. Nano enabled drug delivery has already been successful in delivering drugs to specific tissues within the body, and promises capabilities that will enhance drug penetration into cells, as well as other means to improve drug activity. A very promising prospect of nanoparticles is its use in targeted drug delivery and also “multi-targeting”, which is essential in the case of several diseases. Silver nanoparticles due to their potent characteristics such as anti-permeability, anti-tubules formation, anti-vasculature development, bear out them as an effective molecule in inhibiting angiogenesis and also capable of inducing apoptosis by altering the caspase-3 activity leading DNA fragmentation. They have been proven to carry out these anti-angiogenic functions by inhibiting various metabolic pathways mediated by Src, AKT/P13K that are responsible for VEGF and IL1-β induced angiogenesis similar to the commercially developed ocular drugs (TG100801 — Src inhibitor; Ruboxistaurin — PKC b inhibitor). The targeting of neovascularization cells using silver nanoparticles bound to RGD peptides will emerge the use nanosilver as a novel approach in developing cost economic drugs for treating ocular therapies. The evidence of molecular mechanism by which, silver nanoparticles bound with RGD peptides target the $\alpha_V\beta_3$ integrin

receptors that are over expressed and serve as a marker to the angiogenic cells, confirms the targeting mechanism of the nano drug to the neovascularized cells unaffecting the normal cells. Use of PGA for encapsulation of silver nanoparticles leads to a controlled release of the silver nanoparticles at a single dosage avoiding frequent treatments. Its further inhibitory activity of the pathways leading to angiogenesis within the cell proposes a defined mechanism by which they act. The above evidences archaize that biologically synthesized AgNPs are cost economic swap to the cost effective ocular drugs. Their targeting capability to the neovascularized cells through peptide targeting affirm them as *a trove of nanomedicine* that will certainly tribute to the development of therapeutic advances, serving as a fortunate to ocular therapies.

10. Future directions

There is an enormous interest in exploiting nanoparticles in various biomedical applications since their size scale is similar to that of biological molecules (e.g., proteins and DNA) and structures (e.g., viruses and bacteria). It is likely that the next shift in the treatment of retinal angiogenesis will be towards prolonged intravitreal delivery of current anti-VEGF agents, rather than the replacement of VEGF as the central target in the treatment of ocular angiogenesis. As nanotechnology is undergoing explosive expansion in many areas, even poorer developing countries have also decided that this new technology could represent a considered investment in future economic and social well-being that they cannot ignore. The eye is a very suitable organ for developing novel nanomedicines. Nanoscientists hence should focus their efforts on nano-ophthalmology. In the future transporter/receptor targeted nanoparticles may play a crucial role in drug delivery and release. Our studies on AgNPs effect over angiogenesis make a significant impact in treating common causes of blindness such as PDR and AMD. Further studies on size controlled effect of silver nanoparticles and other down streaming pathways, by which they themselves act as a drug, by controlled release and targeting, will surely signify the role of AuNPs for effective and economic treatments in ocular therapies.

Acknowledgements

Prof. G. Sangiliyandi was supported by grants from Council of Scientific and Industrial Research (CSIR), New Delhi (Grant No: 37/0347), Department of Science and Technology (Grant No: SR/FT/L-118/2006), Department Of Biotechnology (Grant No: BT/PR770/Med/14/1065/2006, BT/PR10211/BCE/08/615/2007), Indian Council of Medical Research (ICMR) (Grant No: 53/5/2006-BMS), and the Government of India, for his various research works supporting this review. Kalimuthu Kalishwaralal is thankful to Council of Scientific and Industrial Research (CSIR), New Delhi for the award of Senior Research Fellowship (142070/2K9/1). The authors also wish thanks to all the members of GS lab for their immense support in completing this review a great deed.

References

[1] L.P. Aiello, Angiogenic pathways in diabetic retinopathy, N. Engl. J. Med. 353 (2005) 839–841.
[2] P. Lee, C.C. Wang, A.P. Adamis, Surv. Ophthalmol. 43 (1998) 245–269.
[3] P.A. Campochiaro, Retinal and choroidal neovascularization, J. Cell. Physiol. 184 (2000) 301–310.
[4] B.C. Polak, A.F. Casparie, Ophthalmological complications of diabetes mellitus and the implementation of the St. Vincent Declaration in The Netherlands, Ned. Tijdschr. Geneeskd. 137 (34) (1993) 1729–1730.
[5] J. Cunha-Vaz, Diabetic macular edema, Eur. J. Ophthalmol. 8 (1998) 127–130.
[6] W. Risau, Angiogenesis and endothelial cell function, Arzneimittelforschung 44 (1994) 1141–1146.
[7] P. Carmeliet, R.K. Jain, Angiogenesis in cancer and other diseases, Nature 407 (2000) 249–257.
[8] Paul Bishop, Hypoxia, angiogenesis and proliferative diabetic retinopathy, Endocr. Abstracts 15 (2008) S19.
[9] U. Schmidt-Erfurth, S. Michels, I. Barbazetto, H. Laqua, Photodynamic effects on choroidal neovascularization and physiological choroid, Invest. Ophthalmol. Vis. Sci. 43 (2002) 830–841.
[10] N. Kwak, N. Okamoto, J.M. Wood, P.A. Campochiaro, VEGF is major stimulator in model of choroidal neovascularization, Invest. Ophthalmol. Vis. Sci. 41 (2000) 3158–3164.
[11] J. Fontenot, J. Rasmussen, L. Williams, J. Dooley, A. Farr, A. Rudensky, Regulatory T cell lineage specification by the forkhead transcription factor Foxp3, Immunity 22 (3) (2005) 329–341.
[12] M. Porta, F. Bandello, Diabetic retinopathyA clinical update, Diabetologia 45 (12) (2002) 1617–1634.
[13] V.H.L. Lee, J.R. Robinson, Topical ocular drug delivery: recent developments and future challenges, J. Ocul. Pharmacol. Ther. 2 (1) (1986) 67–108.
[14] S. Zhang, Z. Bian, C. Gu, Preparation of anti-human cardiac troponin I immunomagnetic nanoparticles and biological activity assays, Colloids Surf. B Biointerfaces 55 (2) (2007) 143–148.
[15] M.A. Dobrovolskaia, P. Aggarwal, J.B. Hall, S.E. McNeil, Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution, Mol. Pharm. 5 (4) (2008) 487–495.
[16] S.K. Sahoo, F. Dilnawaz, S. krishnakumar, Nanotechnology in ocular drug delivery, Drug Discov. Today 13 (2008) 144–151.
[17] I. Brigger, J. Morizet, G. Aubert, H. Chacun, M.J.T. Lacombe, P. Couvreur, G. Vassal, Poly(ethylene glycol)-coated hexadecylcyanoacrylate nanospheres display a combined effect for brain tumor targeting, J. Pharmacol. 303 (3) (2002) 928–936.
[18] G.F. Paciotti, D.G.I. Kingston, L. Tamarkin, Colloidal gold nanoparticles: a novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors, Drug Dev. Res. 67 (1) (2006) 47–54.
[19] M. Moghimi, A.C. Hunter, J.C. Murray, Nanomedicine: current status and future prospects, FASEB J. 19 (2005) 311–330.
[20] P. Mukherjee, R. Bhattacharya, P. Wang, L. Wang, S. Basu, J.A. Nagy, Antiangiogenic properties of gold nanoparticles, Clin. Cancer. Res. 11 (9) (2005) 3530–3534.
[21] R. Bhattacharya, P. Mukherjee, Biological properties of "naked" metal nanoparticles, Adv. Drug. Deliv. Rev. 60 (2008) 1289–1306.
[22] M.J. Folkman, D.M. Long, F.F. Becker, Growth and metastasis of tumor in organ culture, Cancer 16 (1963) 453–467.
[23] J. Folkman, Tumor angiogenesis: therapeutic implications, N Engl J Med. 285 (1971) 1182–1186.
[24] M.A. Gimbrone, S.B. Leapman, R.S. Cotran, J. Folkman, Tumor dormancy in vivo by prevention of neovascularization, J. Exp. Med. 136 (1972) 261–276.
[25] S. Brem, H. Brem, J. Folkman, D. Finkelstein, G. Patz, Prolonged tumor dormancy by prevention of neovascularization in the vitreous, Cancer Res. 36 (1976) 2807–2812.
[26] J. Folkman, K. Watson, D. Ingber, D. Hanahan, Induction of angiogenesis during the transition from hyperplasia to neoplasia, Nature 339 (1989) 58–61.
[27] L. Holmgren, M.S. O'Reilly, J. Folkman, Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression, Nat. Med. 1 (1995) 149–153.
[28] D. Ribatti, Judah Folkman, a pioneer in the study of angiogenesis, Angiogenesis 11 (2008) 3–10.
[29] K. Guillemin, M.A. Krasnow, The hypoxic response: huffing and HIFing, Cell 89 (1997) 9–12.
[30] P. Carmeliet, VEGF gene therapy: stimulating angiogenesis or angiomagenesis? Nat. Med. 6 (2000) 1102–1103.
[31] M.J. Cross, W.L. Claesson, FGF and VEGF function in angiogenesis: signaling pathways, biological responses and therapeutic inhibition, Trends Pharmacol. Sci. 22 (2001) 201–207.
[32] M.K. Van den Enden, J.R. Nyengaard, E. Ostrow, J.H. Burgan, J.R. Williamson, Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism. Implications for diabetic retinopathy, Invest. Ophthalmol. Vis. Sci. 36 (1995) 1675–1685.
[33] Y. Du, C.M. Miller, Hyperglycemia increases mitochondrial superoxide in retina and retinal cells, Free Radic. Biol. Med. 35 (2003) 1491–1499.
[34] R.N. Frank, Diabetic retinopathy, N. Engl. J. Med. 350 (2004) 48–58.
[35] R.E. Gilbert, D. Vranes, Vascular endothelial growth factor and its receptors in control and diabetic rat eyes, Lab. Invest. 78 (1998) 1017–1027.
[36] H.P. Hammes, J. Lin, Upregulation of the vascular endothelial growth factor/vascular endothelial growth factor receptor system in experimental background diabetic retinopathy of the rat, Diabetes 47 (1998) 401–406.
[37] J.W. Miller, A.P. Adamis, Vascular endothelial growth factor in ocular neovascularization and proliferative diabetic retinopathy, Diabetes Metab. Rev. 13 (1997) 37–50.
[38] N. Ferrara, Molecular and biological properties of vascular endothelial growth factor, J. Mol. Med. 77 (1999) 527–543.
[39] V. Vincenti, C. Cassano, M. Rocchi, G. Persico, Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3, Circulation 93 (1996) 1493–1495.
[40] E. Tischer, R. Mitchell, T. Hartman, M. Silva, D. Gospodarowicz, J.C. Fiddes, J.A. Abraham, The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing, J. Biol. Chem. 266 (1991) 11947–11954.
[41] E. Duh, L.P. Aiello, Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox, Diabetes 48 (1999) 1899–1906.
[42] I.C. Michaelson, Vascular morphogenesis in the retina of the cat, J. Anat. 82 (1948) 167–174.

[43] D.R. Senger, S.J. Galli, et al., Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid, Science 219 (1983) 983–985.
[44] P.J. Keck, S.D. Hauser, et al., Vascular permeability factor, an endothelial cell mitogen related to PDGF, Science 246 (1989) 1309–1312.
[45] R.L. Avery, J. Pearlman, Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy, Ophthalmology 113 (2006) 1695e1–169515.
[46] C. Haritoglou, D. Kook, Intravitreal bevacizumab (Avastin) therapy for persistent diffuse diabetic macular edema, Retina 26 (2006) 999–1005.
[47] R. Jorge, R.A. Costa, et al., Intravitreal bevacizumab (Avastin) for persistent new vessels in diabetic retinopathy (IBEPE study), Retina 26 (2006) 1006–1013.
[48] C. Starita, M. Patel, et al., Vascular endothelial growth factor and the potential therapeutic use of pegaptanib (macugen) in diabetic retinopathy, Dev. Ophthalmol. 39 (2007) 122–148.
[49] J. Ideno, H. Mizukami, et al., Prevention of diabetic retinopathy by intraocular soluble flt-1 gene transfer in a spontaneously diabetic rat model, Int. J. Mol. Med. 19 (2007) 75–79.
[50] S. Ishida, T. Usui, et al., VEGF164 is proinflammatory in the diabetic retina, Invest. Ophthalmol. Vis. Sci. 44 (2003) 2155–2162.
[51] T. Qaum, Q. Xu, et al., VEGF-initiated blood-retinal barrier breakdown in early diabetes, Invest. Ophthalmol. Vis. Sci. 42 (2001) 2408–2413.
[52] A.P. Adamis, J.W. Miller, et al., Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy, Am. J. Ophthalmol. 118 (1994) 445–450.
[53] L.P. Aiello, R.L. Avery, et al., Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders, N. Engl. J. Med. 331 (1994) 1480–1487.
[54] J. Pe'er, D. Shweiki, et al., Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases, Lab. Invest. 72 (1995) 638–645.
[55] N. Okamoto, T. Tobe, et al., Transgenic mice with increased expression of vascular endothelial growth factor in the retina: a new model of intraretinal and subretinal neovascularization, Am. J. Pathol. 151 (1997) 281–291.
[56] T.D. Cohen, L.W. Nahari, et al., Interleukin 6 induces the expression of vascular endothelial growth factor, J. Biol. Chem. 271 (1996) 736–741.
[57] D.L. Goad, J. Rubin, et al., Enhanced expression of vascular endothelial growth factor in human SaOS-2 osteoblast-like cells and murine osteoblasts induced by insulin-like growth factor, I, Endocrinology 137 (1996) 2262–2268.
[58] J. Li, M.A. Perrella, et al., Induction of vascular endothelial growth factor gene expression by interleukin-1 beta in rat aortic smooth muscle cells, J. Biol. Chem. 270 (1995) 308–312.
[59] L. Pertovaara, A. Kaipainen, et al., Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells, J. Biol. Chem. 269 (1994) 6271–6274.
[60] M. Ryuto, M. Ono, et al., Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1, J. Biol. Chem. 271 (1996) 28220–28228.
[61] J. Stone, A. Itin, et al., Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia, J. Neurosci. 15 (1995) 4738–4747.
[62] S.G. Robbins, J.R. Conaway, et al., Detection of vascular endothelial growth factor (VEGF) protein in vascular and non-vascular cells of the normal and oxygen-injured rat retina, Growth Factors 14 (1997) 279–295.
[63] L.P. Aiello, J.M. Northrup, et al., Hypoxic regulation of vascular endothelial growth factor in retinal cells, Arch. Ophthalmol. 113 (1995) 1538–1544.
[64] H. Ida, T. Tobe, et al., RPE cells modulate subretinal neovascularization, but do not cause regression in mice with sustained expression of VEGF, Invest. Ophthalmol. Vis. Sci. 44 (2003) 5430–5437.
[65] D.G. Morrison, M. Aschner, et al., Vascular endothelial growth factor response to insulin-like growth factor in normoxic and hypoxic cell culture, Invest. Ophthalmol. Vis. Sci. 48 (2007) 1735.
[66] A.P. Levy, N.S. Levy, et al., Post-transcriptional regulation of vascular endothelial growth factor by hypoxia, J. Biol. Chem. 271 (1996) 2746–2753.
[67] C.M. Brennan, J.A. Steitz, HuR and mRNA stability, Cell. Mol. Life Sci. 58 (2001) 266–277.
[68] S. Robinow, K.M. Campos, et al., The elav gene product of *Drosophila*, required in neurons, has three RNP consensus motifs, Science 242 (1988) 1570–1572.
[69] S.A. Vinores, N.L. Derevjanik, et al., Vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGFbeta), and interleukin-6 (IL-6) in experimental herpesvirus retinopathy: association with inflammation and viral infection, Histol. Histopathol. 16 (2001) 1061–1071.
[70] J.S. Penn, A. Madan, R.B. Caldwell, M. Bartoli, R.W. Caldwell, M.E. Hartnett, Vascular endothelial growth factor in eye disease, Prog. Retin. Eye Res. 27 (2008) 331–371.
[71] T. Alon, I. Hemo, et al., Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity, Nat. Med. 1 (1995) 1024–1028.
[72] N. Ferrara, T. Davis-Smyth, The biology of vascular endothelial growth factor, Endocr. Rev. 18 (1997) 4–25.
[73] H.P. Gerber, V. Dixit, et al., Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells, J. Biol. Chem. 273 (1998) 13313–13316.
[74] H.P. Gerber, A. McMurtrey, et al., Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 30-kinase/ Akt signal transduction pathway. Requirement for Flk-1/KDR activation, J. Biol. Chem. 273 (1998) 30336–30343.
[75] A. Moritz, Konerding ocular angiogenesis: translating preclinical indications to successful clinical development, Expert Opin. Ther. Targets 8 (2004) 255–258.
[76] D. Sherris, Ocular drug development — future directions, Angiogenesis 10 (2007) 71–76.
[77] O.A. Anderson, J.W.B Bainbridge, D.T Shima, Delivery of anti-angiogenic molecular therapies for retinal disease, doi:10.1016/j.drudis.2010.02.004.
[78] R.N. Frank, R.H. Amin, et al., Basic fibroblast growth factor and vascular endothelial growth factor are present in epiretinal and choroidal neovascular membranes, Am. J. Ophthalmol. 122 (1996) 393–403.
[79] R.N. Frank, Potential new medical therapies for diabetic retinopathy: protein kinase C inhibitors, Am. J. Ophthalmol. 133 (2002) 693–698.
[80] H.R. Coleman, C.C. Chan, F.L. Ferris III, E.Y. Chew, Lancet 372 (2008) 1835–1845.
[81] A.J. Brucker, Age-related macular degeneration, Retina 29 (2009) S2–S4.
[82] A. Hoeben, B. Landuyt, M. Highley, H. Wildiers, A.T.V. Oosterom, E.A. Debruijn, Vascular endothelial growth factor and angiogenesis, Pharmacol. Rev. 56 (2004) 549–580.
[83] A. Hoeben, B. Landuyt, M. Highley, H. Wildiers, A.T.V. Oosterom, E.A. Debruijn, Vascular endothelial growth factor and angiogenesis, Pharmacol. Rev. 56 (2004) 549–580.
[84] A. Ewald, K. Susanne, R.G. Thull, U. Gbureck, Antimicrobial titanium/silver PVD coatings on titanium, BioMed. Eng. OnLine 5 (2006) 22.
[85] L. Sintubin, W.D. Windt, J. Dick, J. Mast, D. Van der Ha, W. Verstraete, N. Boon, Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles, Appl. Microbiol. Biotechnol. 84 (2009) (2009) 741–749.
[86] S. Ram Kumar Pandian, V. Deepak, K. Kalishwaralal, P. Viswanathan, S. Gurunathan, Mechanism of bactericidal activity of silver nitrate — a concentration dependent bi-functional molecule, Br. Jr. Micro. (2010) 41. ISSN 1517-8382.
[87] K. Kalishwaralal, V. Deepak, S. Ramkumarpandian, H. Nellaiah, G. Sangiliyandi, Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Bacillus licheniformis*, Mater. Lett 62 (2008) 4411–4413.
[88] K. Kalimuthu, R. Suresh Babu, D. Venkataraman, M. Bilal, S. Gurunathan, Biosynthesis of silver nanocrystals by *Bacillus licheniformis*, Colloid Surf B: Bioint. 65 (2008) 150–153.
[89] R. Vaidyanathan, K. Kalishwaralal, S. Gopalram, S. Gurunathan, Nanosilver—the burgeoning therapeutic molecule and its green synthesis, Biotechnol. Adv. 27 (2009) 924–937.
[90] A.E. Nel, L. Maumldler, D. Velegol, T. Xia, E.M.V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, Understanding biophysicochemical interactions at the nano-bio interface, Nat. Nanotechnol 4 (2009) 543–557.
[91] K. Kawata, M. Osawa, S. Okabe, Non-cytotoxic silver nanoparticle–polysaccharide nanocomposites with antimicrobial activity, Environ. Sci. Technol. 43 (2009) 6046–6051.
[92] S. Gurunathan, K.J. Lee, K. Kalishwaralal, S. Sheikpranbabu, R. Vaidyanathan, S.H. Eom, Antiangiogenic properties of silver nanoparticles, Biomaterials 30 (2009) 6341–6350.
[93] H.A. Pershadsingh, D.M. Moore, PPARγ agonists: potential as therapeutics for neovascular retinopathies, PPAR Res. (2008) 164273.
[94] A. Hoeben, B. Landuyt, M. Highley, H. Wildiers, A.T.V. Oosterom, E.A. Debruijn, Vascular endothelial growth factor and angiogenesis, Pharmacol. Rev. 56 (2004) 549–580.
[95] Q.D. Nguyen, S.M. Shah, G. Hafiz, E. Quinlan, J. Sung, K. Chu, J.M. Cedarbaum, P.A. Campochiaro, A phase I trial of an IV-administered vascular endothelial growth factor trap for treatment in patients with choroidal neovascularization due to age-related macular degeneration, Ophthalmology 113 (2006) 1522.e1–1522.e14.
[96] X.B. Xia, S.Q. Xiong, W.T. Song, J. Luo, Y.K. Wang, R.R. Zhou, Inhibition of retinal neovascularization by siRNA targeting $VEGF_{165}$, Mol. Vis. 14 (2008) 1965–1973.
[97] B. Elayappan, H. Ravinarayannan, S.P.B. SardarPasha, K.J. Lee, S. Gurunathan, PEDF inhibits VEGF- and EPO-induced Angiogenesis in retinal endothelial cells through interruption of PI3K/Akt phosphorylation, 12 (2009) 313–324.
[98] K. Kalishwaralal, E. Banumathi, S.B.R.K. Pandian, V. Deepak, J. Muniyandi, S.H. Eom, G. Sangiliyandi, Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells, Colloid Surfs B Biointerfaces 73 (2009) 51–57.
[99] S. Sheikpranbabu, K. Kalishwaralal, D. Venkataraman, S.H. Eom, J. Park, S. Gurunathan, Silver nanoparticles inhibit VEGF-and IL-1-induced vascular permeability via Src dependent pathway in porcine retinal endothelial cells, J. Nanobiotechnol. 8 (2009).
[100] S. Sheikpranbabu, K. Kalishwaralal, K.J. Lee, R. Vaidyanathan, S.H. Eom, S. Gurunathan, The inhibition of advanced glycation end-produ.cts-induced retinal vascular permeability by silver nanoparticles, Biomaterials 31 (2010) 1318–1329.
[101] D.R. Senger, S.J. Galli, A.M. Dvorak, C.A. Perruzzi, V.S. Harvey, H.F. Dvorak, Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid, Science 219 (1983) 983–985.
[102] H.F. Dvorak, M. Detmar, K.P. Claffey, J.A. Nagy, L. Vandewater, D.R. Senger, Vascular permeability factor/vascular endothelial growth factor: an important mediator of angiogenesis in malignancy and inflammation, Int. Arch. Allergy Immunol. 107 (1995) 233–235.
[103] R.B. Caldwell, M. Bartoli, M.A. Behzadian, A.E.B. El-Remessy, M. Al-Shabrawey, D.H. Platt, R.W. Caldwell, Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives, Diabetes Metab. Res. Rev. 19 (2003) 442–455.
[104] S.R. Boyd, I. Zachary, U. Chakravarthy, G.J. Allen, G.B. Wisdom, I.A. Cree, J.F. Martin, P.G. Hykin, Correlation of increased vascular endothelial growth factor

with neovascularization and permeability in ischemic central vein occlusion, Arch. Ophthalmol. 120 (2002) 1644–1650.
[105] T. Qaum, Q. Xu, A.M. Joussen, M.W. Clemens, W. Qin, K. Miyamoto, H. Hassessian, S.J. Wiegand, J. Rudge, G.D. Yancopoulos, A.P. Adamis, VEGF-initiated blood-retinal barrier breakdown in early diabetes, Invest. Ophthalmol. Vis. Sci. 42 (2001) 2408–2413.
[106] D.A. Antonetti, A.J. Barber, S. Khin, E. Lieth, J.M. Tarbell, T.W. Gardner, Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells, Diabetes 47 (1998) 1953–1959.
[107] D. Amo, E.M., A. Urtti, Current and future ophthalmic drug delivery systems. A shift to the posterior segment, Drug Discov. Today 13 (2008) 135–143.
[108] S.J. DeNardo, P.A. Burke, B.R. Leigh, R.T. O'Donnell, L.A. Miers, L.A. Kroger, S.L. Goodman, S. Matzku, A. Jonczyk, K.R. Lamborn, G.L. DeNardo, Neovascular targeting with cyclic RGD peptide (cRGDf-ACHA) to enhance delivery of radioimmunotherapy, Cancer Biother. Radiopharm. 15 (1) (2000) 71–79.
[109] B. Riecke, E. Chavakis, R.G. Bretzel, T. Linn, K.T. Preissner, M. Brownlee, H.P. Hammes, Topical application of integrin antagonists inhibits proliferative retinopathy, Horm. Metab. Res. 33 (2001) 307–311.
[110] C. Zhan, B. Gu, C. Xie, J. Li, Y. Liu, W. Lu, Cyclic RGD conjugated poly(ethylene glycol)-co-poly(lactic acid) micelle enhances paclitaxel anti-glioblastoma effect, J. Control. Release (2010), doi:10.1016/j.jconrel.2009.12.020.
[111] Schmidt, B. Robert, V. Garambois, L. Rocheblave, J. Martinez, A. Pèlegrin, F. Cavelier, E. Vivès, A RGD tetracyclopeptide as a new tool for specific tumor cell targeting, "Peptides 2008", Helsinki (Finlande), 2008.
[112] E. Chavakis, B. Riecke, J. Lin, T. Linn, R.G. Bretzel, K.T. Preissner, M. Brownlee, H.P. Hammes, Kinetics of integrin expression in the mouse model of proliferative retinopathy and success of secondary intervention with cyclic RGD peptides, Diabetologia 45 (2002) 262–267.
[113] R. Singh, H.E. Grossniklaus, S.J. Kang, H.F. Edelhauser, B.K. Ambati, U.B. Kompella, Intravenous transferrin RGD peptide and dual-targeted nanoparticles enhance anti-VEGF intraceptor gene delivery to laser-induced CNV, Gene Ther. 16 (2009) 645–659.
[114] N.S. Harhaj, D.A. Antonetti, Regulation of tight junctions and loss of barrier function in pathophysiology, Int. J. Biochem. Cell Biol. 36 (2004) 1206–1237.
[115] W. Wu, T. Zhou, A. Berliner, P. Banerjee, S. Zhou, Smart core–shell hybrid nanogels with Ag nanoparticle core for cancer cell imaging and gel shell for pH-regulated drug delivery, Chem. Mater. 22 (2010) 1966–1976.
[116] K. Sonaje, Y.J. Chen, H.L. Chen, S.P. Wey, J.H. Juang, H.N. Nguyen, C.W. Hsu, K.J. Lin, H.W. Sung, Enteric-coated capsules filled with freeze-dried chitosan /poly(g-glutamic acid) nanoparticles for oral insulin delivery, Biomaterials, doi:10.1016/j.biomaterials.2010.01.042.
[117] H. Kimura, Y. Ogura, Biodegradable systems, Intraocular Drug Delivery, Inform. Healthc., 1, 2006, pp. 175–192.
[118] D. Lavinsky, et al., Phase I/II study of intravitreal triamcinolone acetonide microspheres for treatment of diffuse diabetic macular edema unresponsive to conventional laser photocoagulation treatment, Invest. Ophthalmol. Vis. Sci. 49 (2008) 2698.
[119] B. Manocha, A. Margaritis, Production and characterization of γ-polyglutamic acid nanoparticles for controlled anticancer drug release, Crit. Rev. Biotechnol. 28 (2008) 83–99.
[120] L. Baert, G. van't Klooster, W. Dries, et al., Development of a long-acting injectable formulation with nanoparticles of rilpivirine (tmc278) for HIV treatment, Eur. J. Pharm. Biopharm. 72 (3) (2009) 502–508.
[121] S. Okamoto, M. Matsuura, T. Akagi, M. Akashi, T. Tanimoto, T. Ishikawa, M. Takahashi, K. Yamanishi, Y. Mori, Poly(glutamic acid) nano-particles combined with mucosal influenza virus hemagglutinin vaccine protects against influenza virus infection in mice, Vaccine 27 (2009) 5896–5905.
[122] T.Y. Lee, Y.H. Kim, S.W. Yoon, J.C. Choi, J.M. Yang, C.J. Kim, J.T. Schiller, M.H. Sung, H. Poo, Oral administration of poly-gamma-glutamate induces TLR4- and dendritic cell-dependent antitumor effect, Cancer Immunol. Immunother. 58 (2009) 1781–1794.
[123] S.F. Peng, M.J. Yang, C.J. Su, H.L. Chen, P.W. Lee, M.C. Wei, H.W. Sung, Effects of incorporation of poly(g-glutamic acid) in chitosan /DNA complex nanoparticles on cellular uptake and transfection efficiency, Biomaterials 30 (2009) 1797–1808.
[124] TAkao1, T Kimura, Y Hirofuji, K Matsunaga, R Imayoshi, J Nagao, T Cho, H Matsumoto, S Ohtono, J Ohno, K Taniguchi, H Kaminishi, A poly(γ-glutamic acid)-amphiphile complex as a novel nanovehicle for drug delivery system, J. Drug Target, doi:10.3109/10611861003599453.
[125] P. Gopinath, S.K. Gogoi, A. Chattopadhyay, S.S. Ghosh, Implications of silver nanoparticle induced cell apoptosis for in vitro gene therapy, Nanotechnology 19 (2008) 075104.
[126] S. Arora, J. Jain, J.M. Rajwade, K.M. Paknikar, Cellular responses induced by silver nanoparticles: in vitro studies, Toxicol. Lett. 30 (2008) 93–100.