ISSN: 2474-9206

Case Report

Journal of Anesthesia & Pain Medicine

Anti-VEGF Injection Therapeutic Methods for Treatment of Age-Related Macular Degeneration (AMD): A Comparison

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Submitted: 2024, Apr 10; **Accepted:** 2024, May 06; **Published:** 2024, May 12

Citation: Mousavi, S. M., Jalilvand, H., Tabesh, H. (2024) Anti-VEGF Injection Therapeutic Methods for Treatment of Age-Related Macular Degeneration (AMD): A Comparison. *J Anesth Pain Med, 9*(2), 01-09.

Abstract

Anti-VEGF injections are one of the therapeutic methods used to treat various eye conditions, including wet age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), retinal vein occlusion (RVO) and diabetic macular oedema (DMO). These injections contain medications that target vascular endothelial growth factor (VEGF), a protein that promotes the growth of abnormal blood vessels in the eye.

During the procedure, an ophthalmologist administers the anti-VEGF medication directly into the vitreous cavity at the back of the eye. The injection is performed using a small needle, and the eye is typically numbed with local anesthesia to minimize discomfort.

The anti-VEGF medication works by blocking the action of VEGF, which helps to reduce the leakage and growth of abnormal blood vessels in the retina. By doing so, it can help preserve central vision and improve visual outcomes for patients with these conditions.

The frequency and duration of anti-VEGF injections may vary depending on the specific eye condition and the individual patient's response to treatment. In some cases, a series of initial loading doses may be followed by maintenance injections at regular intervals. The treatment plan is typically determined by the ophthalmologist based on the patient's needs and the specific characteristics of their eye condition.

Here, in this review, we are providing a comparison among different usual treatment methods and Anti-VEGF drugs and by a look for the future possible methods, the prospective methods could be predicted.

Keywords: AMD, Bevacizumab, Aflibercept, Nanocarriers, Hydrogels

1. Introduction

As the population around the world ages, more and more people are at risk of chronic eye diseases, aging processes, and eye and systemic co-morbidities. Age-related macular degeneration (AMD), which is non-exudative or ‘‘dry’’ in early stages and may convert to exudative or ‘‘wet’’ in later stages (neovascular AMD, nAMD), is characterized by progressive loss of central vision that can become severe.

Neovascular AMD (nAMD), also known as wet or exudative AMD is characterized by the growth of new, abnormal vasculature from the choriocapillaris extending into the retina, threatening the photoreceptors or retinal pigment epithelium (RPE). Exudation, fluid accumulation and haemorrhages from the vessels can result in vision loss, via RPE detachment or subretinal fibrosis if not promptly treated.

This disease is mostly untreatable and only controllable. Since the main cause of this illness is the abnormal growth of vasculature of the retina, it is controlled by stopping the growth. In other words, controlling the growth by injecting Anti-VEGF into the eyeball. By injecting the anti-VEGF factors into the retin area, the vasclature growth stops for a while and help the patient to have a better sight.

Anti-VEGF injections are one of the therapeutic methods used to treat various eye conditions, including wet age-related macular

degeneration (AMD), proliferative diabetic retinopathy (PDR), retinal vein occlusion (RVO) and diabetic macular oedema (DMO). These injections contain medications that target vascular endothelial growth factor (VEGF), a protein that promotes the growth of abnormal blood vessels in the eye. During the procedure, an ophthalmologist administers the anti-VEGF medication directly into the vitreous cavity at the back of the eye. The injection is performed using a small needle, and the eye is typically numbed with local anesthesia to minimize discomfort. The anti-VEGF medication works by blocking the action of VEGF, which helps to reduce the leakage and growth of abnormal blood vessels in the retina. By doing so, it can help preserve central vision and improve visual outcomes for patients with these conditions. The frequency and duration of anti-VEGF injections may vary depending on the specific eye condition and the individual patient's response to treatment. In some cases, a series of initial loading doses may be followed by maintenance injections at regular intervals. The treatment plan is typically determined by the ophthalmologist based on the patient's needs and the specific characteristics of their eye condition.

Nowadays, Age-related Macular Degeneration (AMD), is known as the leading cause of the vision loss all over the world. In this disease, age is the most critical and effective factor with the prevalence increasing with age. The genetic scores and genetic factors are also really important and can help in prediction of the development of this disease.

Age-related macular degeneration is a multifactorial disease involving complement, lipids, non-genetic risk factors are smoking and low dietary intake of antioxidants (zinc and carotenoids). Early to late stage disease can be slowed with high doses of zinc and antioxidant vitamin preparations. Anti-vascular endothelial growth factor therapy (eg, ranibizumab, aflibercept, or bevacizumab) is highly effective in the treatment of neovascular age-related macular degeneration and significantly reduced the incidence of visual impairment currently, there are no proven treatments for atrophic disease, but there are several substances are studied in clinical trials. Risk factor modification, personalized medicine targeting specific pathways, newer anti-vascular endothelial growth factor factors or other substances and restorative treatment.

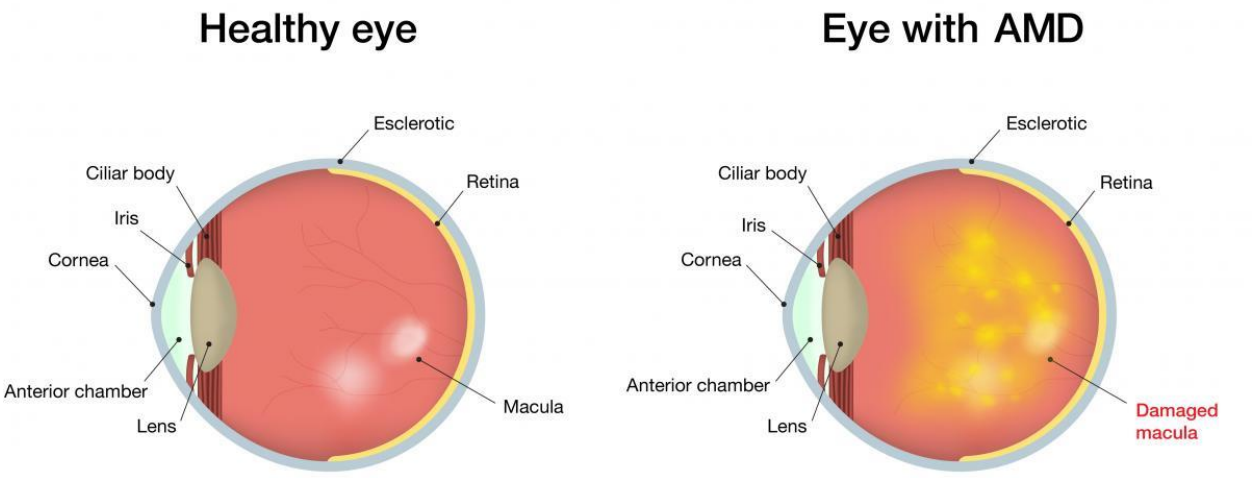


Figure 1: Schematic of a normal eye versus eye with Age-related Macular Degeneration (AMD)

Mechanism of Action

Vascular Endothelial Growth Factor (VEGF) is known to be one of the main regulators of Choroidal Neovascularization (CNV), which is the main cause of wet AMD (nAMD). VEGF family contribute of placenta growth factor, VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E. VEGF-A in this family has a critical and main role in developing pathological angiogenesis in both ischemic and inflammatory diseases [1]. Anti-VEGF drugs inhibits the angiogenesis process as their main duty. Some of the Anti-VEGF drugs, inhibit other growth systems.

Treatment resistance to intravitreal anti-VEGF injections is a clinical problem for nAMD, diabetic retinopathy and RVOs. There are several alternative treatments variable success and the choice of the best treatment must be mostly determined based on the clinical scenario. An important note before we begin one of these alternative approaches is that the patient adheres to the prescribed injection times. Some patients cannot tolerate even a few days behind schedule injection, so strict adherence to the injection schedule may be necessary injection every four weeks. After that, many patients can respond to a simple switch Anti-VEGF agents, while others may require more frequent injections or even injections higher doses than in the standard protocol. Apart from this addition intravitreal steroids in the form of either IVTA, DEX, or FA implants appear to be useful mainly for retinal vascular diseases. Laser treatment (in the form of PDT for nAMD or thermal laser for diabetic retinopathy) is useful when steroid therapy fails or is contraindicated due to other patient characteristics such as poorly controlled glaucoma. Research continues to find new treatment options that can provide a much-needed therapeutic agent in the

ongoing battle against these blinding conditions.

The trending Anti-VEGF used in controlling the nAMD are as below:

- Pegaptanib
- Ranibizumab
- Aflibercept
- Brolucizumab
- Bevacizumab

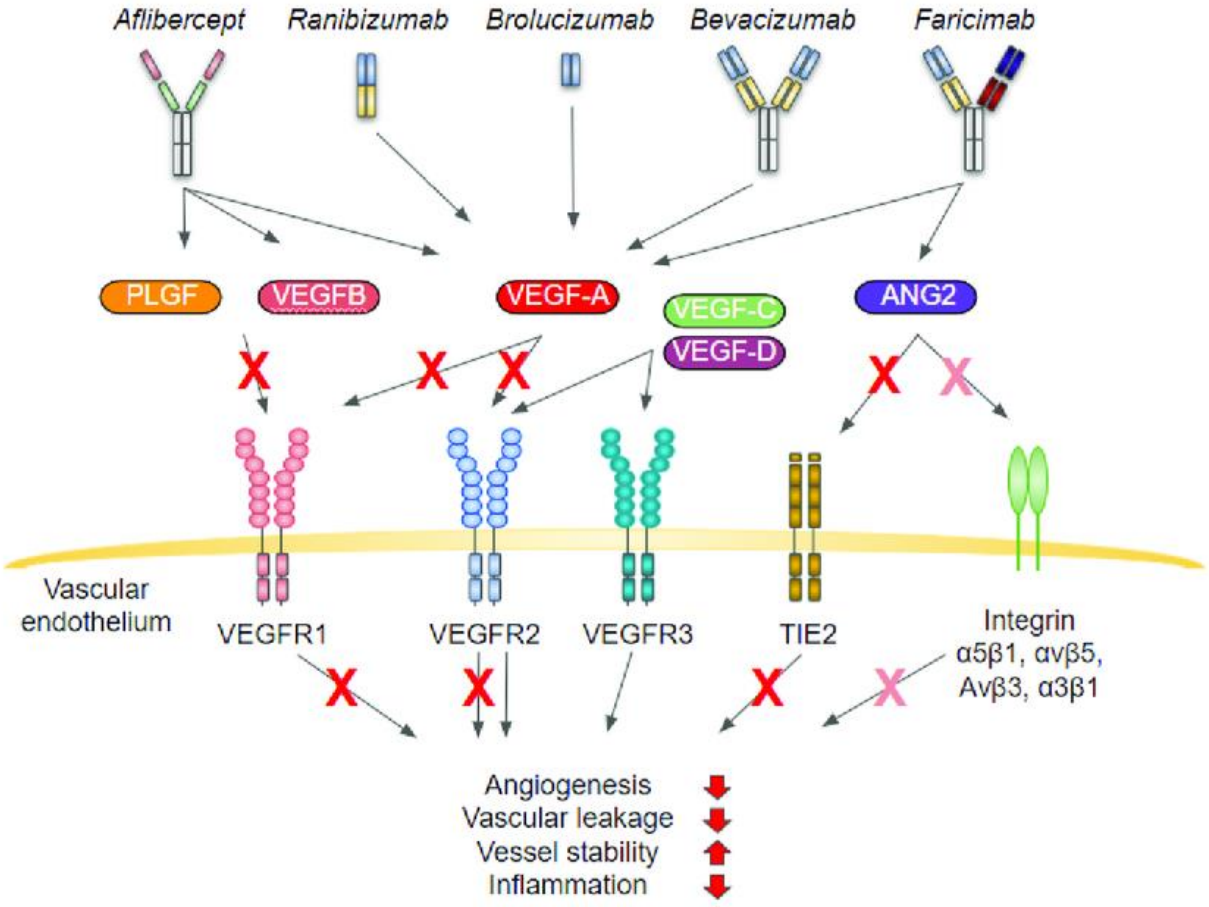


Figure 2: Schematic of mechanism of action of different Anti-VEGF drugs in treating AMD

A shown in figure 2 and the most recent clinical trials, Aflibercept based on its binding ligands, it inhibits most growth factors which can lead to the inhibiotion of abnormal growths as in tumors.

More details are provided in below:

Based on a study in comparison of bevacizumab, Ranibizumab and Aflibercept showed that Intravitreal administration of anti-VEGF antibodies, including bevacizumab, ranibizumab, and aflibercept, is the standard treatment for AMD. These drugs have different charge and molecular weights, which affect their vitreous distribution and elimination. Factors like species involved, physiological and pathological conditions, such as vitrectomy and lensectomy, influence the pharmacokinetic parameters of these drugs. Ranibizumab pharmacokinetics have been tested in monkeys as a non-human primate model. Ocular volume and lens status do not significantly impact the pharmacokinetics and duration of action of anti-VEGF drugs. Aflibercept's vitreous half-life is hypothesized to be nine days, based on its intermediate size between ranibizumab and bevacizumab.

Other studies showing the benefit of using Aflibercept showed Aflibercept is a recombinant fusion protein with a longer duration of action compared to other anti-VEGF drugs, such as bevacizumab and ranibizumab. Aflibercept has a larger molecular weight and size, which may contribute to its extended half-life and sustained release properties. The longer duration of action of aflibercept allows for less frequent injections, reducing the treatment burden for patients with AMD. Aflibercept has a high affinity for multiple VEGF isoforms, including VEGF A, B, and placental growth factor (P1GF), potentially providing broader and more effective inhibition of angiogenesis in AMD. Aflibercept has been shown to have a nine-day aqueous half-life in AMD patients, indicating its potential for sustained release and prolonged therapeutic effect.

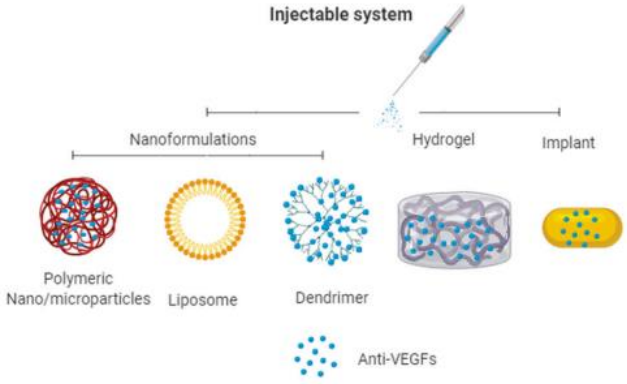
Aflibercept is a medication used in the treatment of age-related

macular degeneration (AMD). It belongs to a class of drugs called anti-VEGF (vascular endothelial growth factor) agents. Aflibercept works by inhibiting the growth of abnormal blood vessels in the retina, which is a key feature of neovascular or "wet" AMD.

Regarding the release profile of aflibercept, it is typically administered as an intravitreal injection, directly into the eye. Once injected, the drug gradually releases into the eye, providing a sustained therapeutic effect. Unlike medications with a burst release profile, aflibercept is designed to have a longer duration of action, allowing for extended treatment intervals.

However, it's important to note that specific formulations and delivery systems for aflibercept may vary depending on the manufacturer and country-specific approvals. Therefore, it's always advisable to consult with a healthcare professional or refer to the product labeling for the most accurate and up-to-date information on aflibercept's release profile and dosing regimen.

In a study done by Seah et al. different types of hydrogels that can be used as delivery systems (specifically as nanocarriers) had been studied and compared. The results as are dragged exactly from the study are as below:



DDSs	Nanoformulations			Bulk system	
Category	Polymeric NPs/MPs	Liposome	Dendrimer	Hydrogel	Implant
Size	1 nm – 1000 μm	25 nm – 2.5 μm	1 nm – 100 nm	Viscous liquid or gel	mm - cm
Drug loading	Encapsulated with the polymeric particle Covalently bond on the surface	Encapsulated in hydrophilic core or conjugate on hydrophilic surface Encapsulated in hydrophobic shell	Trapped inside core or branch cavity Covalently bond on surface	Encapsulated with the hydrogel	Encapsulated in polymeric implant Contained inside the container

Figure 3: Promising strategies for sustained anti-VEGF delivery to the retina

The common treatments for all drugs are direct injection and all the above mentioned drugs are directly administered into the patients' vitreous. Hence, the latest drug delivery systems are trying to eliminate the side effects. In order of that, nanocarriers showed to be effective and promising. Based on the studies in this field, Chitosan nanoparticles loaded by Anti-VEGF, PLGA loaded by drugs and CS/PLGA loaded by drugs showed to be the perfect fits. In brief, the modification process is defined as below:

This work describes a "system within a system", PLGA microparticles containing chitosan-based nanoparticles to improve the loading and sustained intravitreal delivery of ranibizumab. Chitosan-N-acetyl-1-cysteine (CNAC) was synthesized and its synthesis was confirmed by FT-IR and 1H NMR. Chitosan-based nanoparticles consisting of CNAC, CNAC/tripolyphosphate (CNAC/TPP), chitosan, chitosan/TPP (chit/TPP), or chit/TPP-hyaluronic acid (chit/TPP-HA) were incorporated into PLGA microparticles using a modified. w/o/w double emulsion method. Microparticle nanoparticles and final nanoparticles were characterized based on their protein-nanoparticle interaction, size, zeta potential, morphology, protein loading, stability, in vitro release, in vivo antiangiogenic activity, and effect on cell viability. The produced nanoparticles ranged in size from 17 to 350 nm and had zeta potentials from -1.4 to +12 mV. Microscopic imaging revealed spherical nanoparticles on the surface of PLGA microparticles in formulations containing chit/TPP, CNAC, and CNAC/TPP. The blocking efficiency of ranibizumab in the formulations varied between 13 and 69% and was highest for PLGA microparticles containing CNAC nanoparticles. This formulation also showed the slowest release with no initial burst release compared to all other formulations. The addition of TPP to this formulation increased the rate of protein release and decreased the capture efficiency. PLGA microparticles containing Chit/TPP-HA showed the fastest and almost complete release of ranibizumab. All prepared blank particles had no effect on cell viability up to a concentration of 12.5 mg/ml. Ranibizumab released from all formulations maintained its structural integrity

and in vitro activity. The Chit/TPP-HA formulation improved the antiangiogenic effect and may provide a potential biocompatible platform to improve the antiangiogenic effect in combination with ranibizumab. Taken together, PLGA microparticles containing CNAC nanoparticles showed a significantly improved loading and release profile of ranibizumab. This new drug delivery system may have the potential to improve the intravitreal delivery of therapeutic proteins, reducing the frequency, risk and cost of difficult intravitreal injections.

Dexamethasone can affect VEGF expression or function, while bevacizumab targets and binds to VEGF. In this study dexamethasone-loaded poly(D,L-lactide-co-glycolide)/polyethyleneimine nanoparticles (eBev-DPPNs) containing electrostatically conjugated bevacizumab for angiogenic combination therapy in eye diseases was prepared. A novel polynanoparticle consisting of (D), L-lactide-co-glycolide) and polyethyleneimine and loaded the nanoparticles with dexamethasone. Bevacizumab was adsorbed on the nanoparticle surfaces through electrostatic interactions. EBev-DPPNs were evaluated based on their size, polydispersity index, zeta potential, morphology, drug loading, release behavior and stability. The structural stability of bevacizumab on the nanoparticle surface was also analyzed. Angiogenesis was then investigated in the presence of eBev-DPPNs using cell apoptosis, wound healing, Transwell invasion and tube formation assays in human umbilical vein endothelial cells (HUVEC) in vitro and chicken embryonic chorioallantoic membrane assay in vivo. Intravitreal injection of eBev-DPPN was used in a laser-induced rabbit choroidal neovascularization (CNV) model to confirm the role of potential intravitreal applications.Results: eBev-DPPNs were approximately 200 nm in diameter and had a narrow diameter distribution. surface charge was neutral (0.85 ± 0.37 mV), making eBev-DPPNs stable under physiological conditions. Apoptosis, migration, invasion and tube formation assays showed that eBev-DPPNs had good antiangiogenic effects on HUVECs. eBev-DPPNs also exerted a strong inhibitory effect on VEGF secretion by HUVECs. In addition, in vivo chicken embryo chorioallantoic membrane analysis showed that eBev-DPPNs significantly reduced the number of blood vessels. CNV leakage area was reduced in the eBev-DPPNs group in a rabbit CNV model.Conclusion: eBev-DPPNs are a promising new anti-angiogenic drug for potential intravitreal applications such as age-related macular degeneration.

Vascular endothelial growth factor (VEGF) levels have been found to be elevated in several eye diseases. The most commonly used drug for these conditions is bevacizumab, an anti-VEGF drug. For effective treatment of these diseases, sending drugs to the back region is desirable. In this study bevacizumab poly(lactide-co-glycolic acid) (PLGA) nanoparticles (NPs) coated with chitosan (CS) was developed and optimized for sustained and effective delivery to the posterior tissues of the eye. The NPs were prepared by a double emulsion solvent evaporation method and optimized for different variables (i.e., CS concentration, PLGA concentration, polyvinyl alcohol (PVA) concentration, and sonication time) using a 4-factor, 3-level Box-Behnken statistical design. The NPs were characterized by particle size, polydispersity index (PDI), entrapment efficiency (EE) and in vitro release. Transscleral flow was determined through the sclera of the goat, and ocular tolerance was determined by the chorioallantoic membrane method of the chicken egg test. The particle size and PDI of the optimized NPs were 222.28 ± 7.45 nm and 0.19 ± 0.08, respectively. The developed NPs showed an EE of 69.26 ± 1.31% with an extended release profile. The flux was significantly higher, i.e., 0.3204 ± 0.026 μg/cm²/h for NPs compared to the drug solution. Thus, CS-coated PLGA-NPs may be potentially useful as ophthalmic drug carriers for retinal targeting.

In a most recent study in 2020, Jiang et al. developed a sustained release system that helps to release the Anti-VEGF drugs to over a year. In brief, by developing an injectable biodegradable bilayered capsules, they achieved in about a one-year drug release. The description of this method is as follows. Vascular endothelial growth factor (VEGF) is a key regulator of abnormal blood vessel growth. As such, bevacizumab-based inhibition of VEGF has been the clinically adapted strategy to treat colorectal and breast cancers as well as age-related macular degeneration (AMD). However, as the treatment of vascular diseases often requires a high drug concentration for a long period, the burst release of bevacizumab remains a critical limitation in anti-VEGF-based therapies. Maintaining bevacizumab at high concentrations over extended periods remains challenging due to insufficient drug loading capacity and drug-device interactions. The study reported the development of a polymeric based bi-layered capsule that could address these challenges by extending the release over one year, thereby providing an effective platform enabling treatment of chronic vascular diseases. Remarkably, the developed capsules have a bi-layered structure which ensures the structural integrity of the injectable capsules and appropriate diffusion of bevacizumab by providing optimal physical trapping and electrostatic interaction. Meanwhile, the central hollow design enables a higher drug loading to meet the need for long-term release of bevacizumab for several months to one year. Using an in vitro drug release assay, we demonstrated that the bilayered capsule could produce longer-term local drug administration by intravitreal injection compared to previously reported devices. The capsules also present minimal toxicity and maintain anti-VEGF potency, suggesting that our approach may have the potential to treat vascular-related diseases using bevacizumab.

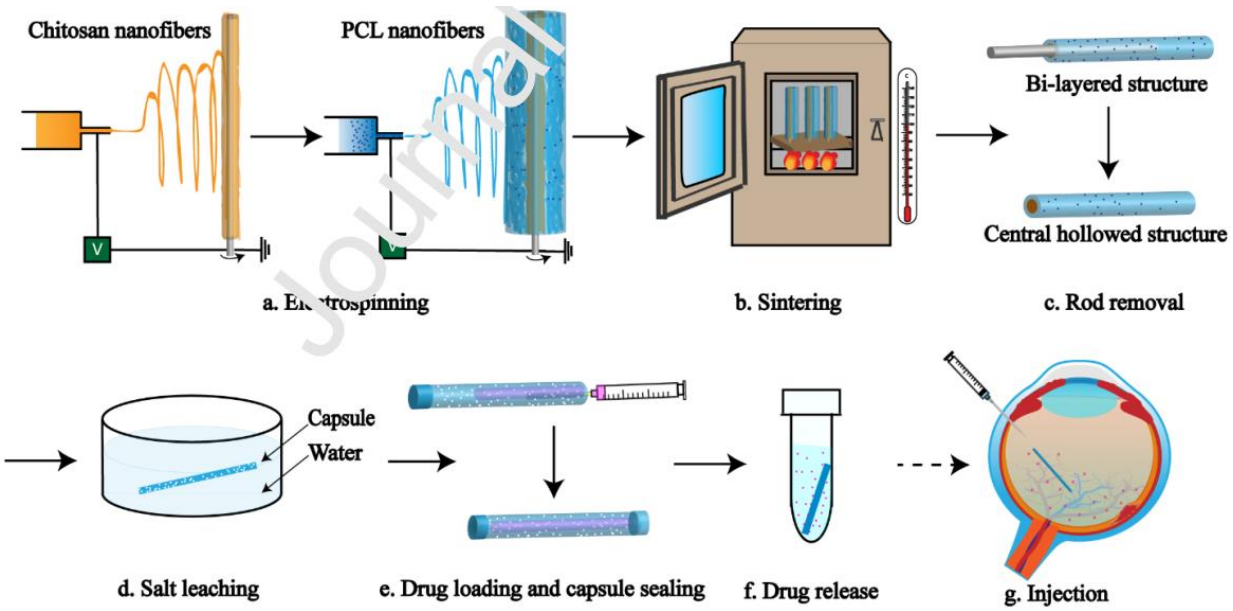


Figure 4: Schematic of bi-layered capsule fabrication process. a) Two layers of chitosan and PCL nanofibers collected on the rotary rod using electrospinning. b) Bi-layered coated rod sintered at 100□C in the vacuum oven for 3 hours. c) Rod removal to create a central hollowed cylinder. d) Porous structure in PCL layer generated by salt leaching. e) Therapeutic loading to the capsule followed by end sealing. f) Drug release in PBS at 37□C. g) Intravitreal injection.

Results & Discussion

The best choice that has already shown more promising results than the others is "Aflibercept". Table 1 shows some of the differences among common Anti-VEGF drugs used to treat nAMD.

Anti-VEGF	Burst release	Molecular weight (kDa)	Half-life in posterior segment of eye	Binding VEGF	Effectiveness duration (week)	Effective dosage
Avastin (bevacizumab)	+	149 kDa	~8 days	VEGF A	2-4	1 mg to 2.5 mg (probably 1.25 mg)
Faricimab	+	150 kDa	~7.5 days	VEGF A, ANG 2	3-4	6 mg
Ranizumab	+	48 kDa	~5 days	VEGF A, IgG1	3-4	0.5 mg (0.05 mL)
Aflibercept	-	115 kDa	~7 days	VEGF a, VEGF B, PLGF	6-8	2 mg (0.05 mL)

Table 1: Comparison of different Anti-VEGF common used drugs to treat Age-related Macular Degeneration (AMD)

There is still a significant clinical demand for sustained delivery systems that target the posterior segment of the eye and provide antiVEGF treatment. However, developing such drug delivery systems is a complex task due to the challenge of encapsulating enough anti-VEGF in a small volume (<0.1 ml) while ensuring its sustained release and bioactivity in vivo. Among various research approaches, non-biodegradable implants have shown the most potential and are closest to obtaining clinical approval. Nevertheless, these implants are associated with complications arising from the surgical implantation procedure. On the other hand, hydrogels have emerged as the most promising option, as recent publications have demonstrated their good biocompatibility and safety. Furthermore, industry-driven clinical trials are on the horizon, further highlighting the potential of hydrogels in this field. The different dosage and duration can be effective on the patients' and their companions' quality of life (QOL) either economically or mentally. Since the current treatments require different and multiple injections per year, enhancing a technology

to gain the sustain, long-duration drug release profile could be a promising and prospective method. Using electrospinning to create microcarriers showed an incredibly great results, hence, it is still on lab-scale and providing such treatment method could not be economically profitable. Nanocarriers on the other hand, showed to be a better choice, since the synthesis process is more affordable and takes less time, also, it is already being used in the pharmaceutical market, so it can be industrial. On the other hand, due to the shorter term duration of degradation in compare to the electrospun matt, it cannot provide as long time as using electrospinning method.

We in this study, suggest the invention of a hybrid Drug Delivery System (DDS) in a combination of the two aforementioned systems in order to get both long-time release profile and an affordable product [2-62].

References

1. Kim, J., Kudisch, M., da Silva, N. R. K., Asada, H., Aya-Shibuya, E., Bloomer, M. M., ... & Desai, T. A. (2018). Long-term intraocular pressure reduction with intracameral polycaprolactone glaucoma devices that deliver a novel anti-glaucoma agent. *Journal of Controlled Release, 269*, 45-51.
2. Michels, S., Rosenfeld, P. J., Puliafito, C. A., Marcus, E. N., & Venkatraman, A. S. (2005). Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration: twelve-week results of an uncontrolled open-label clinical study. *Ophthalmology, 112*(6), 1035-1047.
3. Lau, P., Jenkins, K. S., & Layton, C. (2018). Current evidence for the prevention of endophthalmitis in anti-VEGF intravitreal injections. *Journal of ophthalmology, 2018.*
4. MASON III, J. O., White, M. F., Feist, R. M., Thomley, M. L., Albert, M. A., Persaud, T. O., ... & Vail, R. S. (2008). Incidence of acute onset endophthalmitis following intravitreal bevacizumab (Avastin) injection. *Retina, 28*(4), 564-567.
5. Bhavsar, A. R., Googe, J. M., Stockdale, C. R., Bressler, N. M., Brucker, A. J., Elman, M. J., ... & Diabetic Retinopathy Clinical Research Network. (2009). Risk of endophthalmitis after intravitreal drug injection when topical antibiotics are not required: the diabetic retinopathy clinical research network laser-ranibizumab-triamcinolone clinical trials. *Archives of ophthalmology, 127*(12), 1581-1583.
6. Spooner, K. L., Mhlanga, C. T., Hong, T. H., Broadhead, G. K., & Chang, A. A. (2018). The burden of neovascular age-related macular degeneration: a patient's perspective. *Clinical Ophthalmology,* 2483-2491.
7. Prenner, J. L., Halperin, L. S., Rycroft, C., Hogue, S., Liu, Z. W., & Seibert, R. (2015). Disease burden in the treatment of age-related macular degeneration: findings from a time-and-motion study. *American journal of ophthalmology, 160*(4), 725-731.
8. Wong, W. L., Su, X., Li, X., Cheung, C. M. G., Klein, R., Cheng, C. Y., & Wong, T. Y. (2014). Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet Global Health, 2*(2), e106-e116.
9. Lee, R., Wong, T. Y., & Sabanayagam, C. (2015). Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye and vision, 2*, 1-25.
10. Huang, H., He, J., Johnson, D. K., Wei, Y., Liu, Y., Wang, S., ... & Semba, R. D. (2015). Deletion of placental growth factor prevents diabetic retinopathy and is associated with Akt activation and HIF1α-VEGF pathway inhibition. *Diabetes, 64*(1), 200-212.
11. Wisniewska-Kruk, J., Hoeben, K. A., Vogels, I. M., Gaillard, P. J., Van Noorden, C. J., Schlingemann, R. O., & Klaassen, I. (2012). A novel co-culture model of the blood-retinal barrier based on primary retinal endothelial cells, pericytes and astrocytes. *Experimental eye research, 96*(1), 181-190.
12. Wells, J. A., Glassman, A. R., Ayala, A. R., Jampol, L. M., Aiello, L. P., Antoszyk, A. N., ... & Beck, R. W. (2015). Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *The New England journal of medicine, 372*(13), 1193-1203.
13. Heimes, B., Gunnemann, F., Ziegler, M., Gutfleisch, M., Spital, G., Pauleikhoff, D., & Lommatzsch, A. (2016). Compliance von Patienten mit altersabhängiger Makuladegeneration unter Anti-VEGF-Therapie. *Der Ophthalmologe, 11*(113), 925-932.
14. Radhakrishnan, K., Sonali, N., Moreno, M., Nirmal, J., Fernandez, A. A., Venkatraman, S., & Agrawal, R. (2017). Protein delivery to the back of the eye: barriers, carriers and stability of anti-VEGF proteins. *Drug discovery today, 22*(2), 416-423.
15. Imperiale, J. C., Acosta, G. B., & Sosnik, A. (2018). Polymer-based carriers for ophthalmic drug delivery. *Journal of controlled release, 285*, 106-141.
16. Lee, S. S., Hughes, P., Ross, A. D., & Robinson, M. R. (2010). Biodegradable implants for sustained drug release in the eye. *Pharmaceutical research, 27,* 2043-2053.
17. Kim, Y. C., Chiang, B., Wu, X., & Prausnitz, M. R. (2014). Ocular delivery of macromolecules. *Journal of Controlled Release, 190,* 172-181.
18. Silva, G. R. D., Fialho, S. L., Siqueira, R. C., Jorge, R., & Cunha Júnior, A. D. S. (2010). Implants as drug delivery devices for the treatment of eye diseases. *Brazilian Journal of Pharmaceutical Sciences, 46,* 585-595.
19. Li, F., Hurley, B., Liu, Y., Leonard, B., & Griffith, M. (2012). Controlled release of bevacizumab through nanospheres for extended treatment of age-related macular degeneration. *The open ophthalmology journal, 6,* 54.
20. Sousa, F., Cruz, A., Fonte, P., Pinto, I. M., Neves-Petersen, M. T., & Sarmento, B. (2017). A new paradigm for antiangiogenic therapy through controlled release of bevacizumab from PLGA nanoparticles. *Scientific Reports, 7*(1), 3736.
21. Lu, L., Yaszemski, M. J., & Mikos, A. G. (2001). Retinal pigment epithelium engineering using synthetic biodegradable

polymers. *Biomaterials, 22*(24), 3345-3355.
22. Sun, H., Mei, L., Song, C., Cui, X., & Wang, P. (2006). The in vivo degradation, absorption and excretion of PCL-based implant. *Biomaterials, 27*(9), 1735-1740.
23. Tabesh, H., Jalilvand, H., & Mousavi, S. M. (2024). Anti-VEGF injection therapeutic methods for treatment of Age-related Macular Degeneration (AMD).
24. Bernards, D. A., Bhisitkul, R. B., Wynn, P., Steedman, M. R., Lee, O. T., Wong, F., ... & Desai, T. A. (2013). Ocular biocompatibility and structural integrity of micro-and nanostructured poly (caprolactone) films. *Journal of ocular pharmacology and therapeutics, 29*(2), 249-257.
25. Lance, K. D., Good, S. D., Mendes, T. S., Ishikiriyama, M., Chew, P., Estes, L. S., ... & Desai, T. A. (2015). In vitro and in vivo sustained zero-order delivery of rapamycin (sirolimus) from a biodegradable intraocular device. *Investigative ophthalmology & visual science, 56*(12), 7331-7337.
26. Armani, D. K., & Liu, C. (2000). Microfabrication technology for polycaprolactone, a biodegradable polymer. *Journal of Micromechanics and Microengineering, 10*(1), 80.
27. Mi, F. L., Tan, Y. C., Liang, H. F., & Sung, H. W. (2002). In vivo biocompatibility and degradability of a novel injectable-chitosan-based implant. *Biomaterials, 23*(1), 181-191.
28. Kean, T., & Thanou, M. (2010). Biodegradation, biodistribution and toxicity of chitosan. *Advanced drug delivery reviews, 62*(1), 3-11.
29. prasanth Koppolu, B., Smith, S. G., Ravindranathan, S., Jayanthi, S., Kumar, T. K. S., & Zaharoff, D. A. (2014). Controlling chitosan-based encapsulation for protein and vaccine delivery. *Biomaterials, 35*(14), 4382-4389.
30. George, M., & Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. *Journal of controlled release, 114*(1), 1-14.
31. Lu, Y., Zhou, N., Huang, X., Cheng, J. W., Li, F. Q., Wei, R. L., & Cai, J. P. (2014). Effect of intravitreal injection of bevacizumab-chitosan nanoparticles on retina of diabetic rats. *International journal of ophthalmology, 7*(1), 1.
32. Badiee, P., Varshochian, R., Rafiee-Tehrani, M., Abedin Dorkoosh, F., Khoshayand, M. R., & Dinarvand, R. (2018). Ocular implant containing bevacizumab-loaded chitosan nanoparticles intended for choroidal neovascularization treatment. *Journal of Biomedical Materials Research Part A, 106*(8), 2261-2271.
33. Zhu, Y., Shi, J., Shen, W., Dong, X., Feng, J., Ruan, M., & Li, Y. (2005). Stimuli-responsive controlled drug release from a hollow mesoporous silica sphere/polyelectrolyte multilayer core–shell structure. *Angewandte Chemie International Edition, 44*(32), 5083-5087.
34. Gu, B. K., Park, S. J., Kim, M. S., Kang, C. M., Kim, J. I., & Kim, C. H. (2013). Fabrication of sonicated chitosan nanofiber mat with enlarged porosity for use as hemostatic materials. *Carbohydrate Polymers, 97*(1), 65-73.
35. Cipitria, A., Skelton, A., Dargaville, T. R., Dalton, P. D., & Hutmacher, D. W. (2011). Design, fabrication and characterization of PCL electrospun scaffolds—a review. *Journal of Materials Chemistry, 21*(26), 9419-9453.
36. Chaparro, F. J., Presley, K. F., da Silva, M. A. C., & Lannutti, J. J. (2019). Sintered electrospun polycaprolactone for controlled model drug delivery. *Materials Science and Engineering: C, 99,* 112-120.
37. Nam, J., Johnson, J., Lannutti, J. J., & Agarwal, S. (2011). Modulation of embryonic mesenchymal progenitor cell differentiation via control over pure mechanical modulus in electrospun nanofibers. *Acta biomaterialia, 7*(4), 1516-1524.
38. Chaparro, F. J., Presley, K. F., Coutinho da Silva, M. A., Mandan, N., Colachis, M. L., Posner, M., ... & Lannutti, J. J. (2019). Sintered electrospun poly (ε-caprolactone)–poly (ethylene terephthalate) for drug delivery. *Journal of Applied Polymer Science, 136*(26), 47731.
39. Elzein, T., Nasser-Eddine, M., Delaite, C., Bistac, S., & Dumas, P. (2004). FTIR study of polycaprolactone chain organization at interfaces. *Journal of colloid and interface science, 273*(2), 381-387.
40. Osman, Z., & Arof, A. K. (2003). FTIR studies of chitosan acetate based polymer electrolytes. *Electrochimica Acta, 48*(8), 993-999.
41. Bernards, D. A., Lance, K. D., Ciaccio, N. A., & Desai, T. A. (2012). Nanostructured thin film polymer devices for constant-rate protein delivery. *Nano letters, 12*(10), 5355-5361.
42. Joseph, J. J., Sangeetha, D., & Gomathi, T. (2016). Sunitinib loaded chitosan nanoparticles formulation and its evaluation. *International journal of biological macromolecules, 82,* 952-958.
43. Diop, M., Auberval, N., Viciglio, A., Langlois, A., Bietiger, W., Mura, C., ... & Sigrist, S. (2015). Design, characterisation, and bioefficiency of insulin–chitosan nanoparticles after stabilisation by freeze-drying or cross-linking. *International journal of pharmaceutics, 491*(1-2), 402-408.
44. Xue, M., Hu, S., Lu, Y., Zhang, Y., Jiang, X., An, S., ... & Jiang, C. (2015). Development of chitosan nanoparticles as drug delivery system for a prototype capsid inhibitor. *International Journal of Pharmaceutics, 495*(2), 771-782.
45. El-Shabouri, M. H. (2002). Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. *International journal of pharmaceutics, 249*(1-2), 101-108.
46. Gao, P., Xia, G., Bao, Z., Feng, C., Cheng, X., Kong, M., ... & Chen, X. (2016). Chitosan based nanoparticles as protein carriers for efficient oral antigen delivery. *International journal of biological macromolecules, 91,* 716-723.
47. Bagre, A. P., Jain, K., & Jain, N. K. (2013). Alginate coated chitosan core shell nanoparticles for oral delivery of enoxaparin: in vitro and in vivo assessment. *International journal of pharmaceutics, 456*(1), 31-40.
48. Wang, J., Tan, J., Luo, J., Huang, P., Zhou, W., Chen, L., ... & Deng, D. Y. (2017). Enhancement of scutellarin oral delivery

efficacy by vitamin B12-modified amphiphilic chitosan derivatives to treat type II diabetes induced-retinopathy. *Journal of nanobiotechnology, 15,* 1-17.
49. Huang, Y. C., Chen, J. K., Lam, U. I., & Chen, S. Y. (2014). Preparing, characterizing, and evaluating chitosan/fucoidan nanoparticles as oral delivery carriers. *Journal of Polymer Research, 21,* 1-9.
50. Shi, Y., Jia, L., Du, Q., Niu, J., & Zhang, D. (2018). Surface-modified PLGA nanoparticles with chitosan for oral delivery of tolbutamide. Colloids and Surfaces B: *Biointerfaces, 161*, 67-72.
51. Derakhshandeh, K., & Fathi, S. (2012). Role of chitosan nanoparticles in the oral absorption of Gemcitabine. *International journal of pharmaceutics, 437*(1-2), 172-177.
52. Maity, S., Mukhopadhyay, P., Kundu, P. P., & Chakraborti, A. S. (2017). Alginate coated chitosan core-shell nanoparticles for efficient oral delivery of naringenin in diabetic animals—An in vitro and in vivo approach. *Carbohydrate polymers, 170,* 124-132.
53. Liang, J., Yan, H., Yang, H. J., Kim, H. W., Wan, X., Lee, J., & Ko, S. (2016). Synthesis and controlled-release properties of chitosan/β-Lactoglobulin nanoparticles as carriers for oral administration of epigallocatechin gallate. *Food science and biotechnology, 25,* 1583-1590.
54. Aluani, D., Tzankova, V., Kondeva-Burdina, M., Yordanov, Y., Nikolova, E., Odzhakov, F., ... & Yoncheva, K. (2017). Evaluation of biocompatibility and antioxidant efficiency of chitosan-alginate nanoparticles loaded with quercetin. *International journal of biological macromolecules, 103*, 771-782.
55. Van der Lubben, I. M., Verhoef, J. C., Borchard, G., & Junginger, H. E. (2001). Chitosan for mucosal vaccination. *Advanced drug delivery reviews, 52*(2), 139-144.
56. Casettari, L., & Illum, L. (2014). Chitosan in nasal delivery systems for therapeutic drugs. *Journal of Controlled Release, 190,* 189-200.
57. Illum, L. (2003). Nasal drug delivery—possibilities, problems and solutions. *Journal of controlled release, 87*(1-3), 187-198.
58. Shahnaz, G., Vetter, A., Barthelmes, J., Rahmat, D., Laffleur, F., Iqbal, J., ... & Bernkop-Schnürch, A. (2012). Thiolated chitosan nanoparticles for the nasal administration of leuprolide: bioavailability and pharmacokinetic characterization. *International journal of pharmaceutics, 428*(1-2), 164-170.
59. Ruge, C. A., Kirch, J., & Lehr, C. M. (2013). Pulmonary drug delivery: from generating aerosols to overcoming biological barriers—therapeutic possibilities and technological challenges. *The lancet Respiratory medicine, 1*(5), 402-413.
60. Lytting, E., Nguyen, J., Wang, X., & Kissel, T. (2008). Biodegradable polymeric nanocarriers for pulmonary drug delivery Biodegradable polymeric nanocarriers for pulmonary drug delivery. *Expert Opin. Drug Deliv, 56*, 629-639.
61. Islam, N., & Ferro, V. (2016). Recent advances in chitosan-based nanoparticulate pulmonary drug delivery. *Nanoscale, 8*(30), 14341-14358.
62. Rawal, T., Parmar, R., Tyagi, R. K., & Butani, S. (2017). Rifampicin loaded chitosan nanoparticle dry powder presents an improved therapeutic approach for alveolar tuberculosis. *Colloids and Surfaces B: Biointerfaces, 154,* 321-330.