CHAPTER. 1

INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Amino acids are the privileged class of building blocks in drug designing, as the amino acids are the amine and carboxylic acid functional group-containing organic compounds. Its polymerized forms are protein-building blocks that play a necessary role in the construction and synthesis of hormones, and neurotransmitters and finally have a vital role in body functions. The different amino acids have important role in body functions; like phenylalanine, glutamine and glutamate boost the healthy nervous system, Glycine promote the cell growth, leucine promotes protein synthesis, cysteine act as antioxidant, histidine involve in enzymatic process etc. Amino acid-based polymers have emerged as the new biomaterials in recent years. Polymerization of amino acid via acylation, amidation and esterification etc. yield the biodegradable and biocompatible polymer. Amino acid based polymers produce the biocompatible degradation product are nontoxic and safe, which can be easily eliminated through the metabolic pathway.[1] As an example acylglycine is an metabolic byproduct of amino acid and fatty acid metabolism [2]. Due to presence of functional groups amino acid derived polymer offer the key advantage like, (i) introduction of new imaging, molecular targeting and drug moieties, (ii) modulation of swelling and rheological behavior for target application, (iii) offers improved biological properties like cell proliferation, adhesion and biodegradation, (iv) can improve the thermal and mechanical properties and (v) degradation products are nontoxic and mimic like readily metabolized product from the body. [3] Except glycine all the amino acids are chiral and hence considered as a promising candidate in

development of chiral polymer. Living cells are imprinted with chiral-based signature and chirality shows strong influence in many physiological/ biological events such as protein adsorption, cell adhesion, differentiation, and autophagy etc.[4, 5] The majority of research has demonstrated that although L-type chiral polymers activate the immune system and signaling pathway, D-type chiral polymers promote the cell adhesion, macrophage accumulation as well as anti-inflammatory qualities. [4] In Pharmaceutical chirality is considered as important aspect of drug development because enantiomers of chiral drug shows different pharmacokinetic and pharmacological properties. Like pure enantiomer as compared to racemate can enhance the effectiveness and safety of the treatment.[6]Amino acid polymers offers the various architecture, geometries and sizes. Due to presence functional moieties, it can build the smart polymer like pH responsive, redox responsive, thermoresponsive and self-assembled polymer. Higashi et. al proposed that copolymerization of different amino acid based acrylate monomers can be widely tunned in UCST/LCST behavior in between 18 °C and 73 °C. According to their findings, the Gly-based polymer did not exhibit phase separation, while the Ala-based polymer showed an upper critical solution temperature (UCST) behavior in water below pH 2.0 because of the thermo-reversible hydrogen bonding of the pendent COOH groups. [7] Similarly LCST behavior of PVCL has been induced by addition of tryptophan along with improvement in biocompatibility.[8] The responsive behavior of acid-derived polymers in response to different chemical, physical or biochemical stimuli such as gas, redox-, metal ions, pH, temperature, light, enzyme, glucose, proteins, nucleic acid, DNA, or a combination, and the recent advancements in designing synthesis and biomedical applications are well illustrated. by Burari et. al.[9]

Among the various kind of polymers, proteins are bricks of life made up of amino acids. Although proteins are the polymers and nutritive potential for cells, but have the short coming in the applications due to inconsistency in properties of different batches, disease transmission risk and immune suppression, which restricts use. However, because they are not constrained by the limits of proteins and still have the favorable characteristics of high tissue compatibility, nutritional potential, and tunable mechanical properties, synthetic pendent amino acid-based polymers are more promise as degradable biomaterials. Additionally, due to key features of amino acid based polymers like High biocompatibility, biodegradability, cell adhesion and in proliferative nature towards MSCs and are used in tissue engineering. With due presence of a lot of advantageous properties, the present work is divided in two categories i.e, the development of neuro-regenerative material and the development of anticancer polymer as synergistic delivery systems for the anticancer application. Although there are continuous efforts are made in the development of neuro-regenerative medicines but unfortunately research has not reached the target in terms of clinical success. While, with the continuous increase in the neuro degeneration, traumatic injuries, mental stress associated collapse, brain cancer and associated mortalities, a continuously increasing in the demand for regenerative materials are increasing. Presently very few materials are known for neuro –regenerative, however, amino acid-based materials are negligible. Vascular damage, increased inflammations, oxidative stress and insufficient blood supply are the leading causes of death and neurological disability in stroke/TBI and failure in recovery with the existing treatment options.[10, 11] Few researchers are trying to explore for development of angiogeneic and neurogenic regenerative medicines by using growth factors, however, use of vascular endothelial growth factor (vegf) can arise the new complication in lack of target specificity.

And to the best of our knowledge none of the researchers explored for the development of glycine and glutamate based neurotransmitter mimicking material for development of neuro-regenerative material. Therefore, in the present work we have designed the glycine and glutamate based material and explored for their angiogenic and neurogenic potential under oxidative stress without use of VEGF. Sometime material could be tumorigenic; therefore, the cytotoxic behavior against cancer cells but not against the normal cell is also explored in the present study.

For the development of synergistic anticancer delivery vehicle, we are motivated by bentolia group, where they have mention about the heparanase inhibitory property of amino acid acrylate based polymers; however, limitation of their study is that they have synthesized linear and high molecular weight homopolymer. As heparanase is one of the key enzyme which mostly expressed in aggressive form of cancer and responsible for tumor invasion. In the present work we have developed the anticancer p(NAG-b-A) polymer using computational approach which can irradiate the heparanase driven polymer. It is known that, amino acid based polymers can yield both anionic, cationic and amphiphilic type of polymers and show advantageous characteristic in different kind of biological applications. Similar to how cationic polymers promote the uptake of drugs and siRNA by cells while shielding them from enzymatic breakdown. However, because siRNA has a low charge density, it is difficult for cationic polymers to efficiently condense siRNA. Anionic polymers, however, make excellent candidates for siRNA condensation. and enhanced *in vivo* delivery of siRNA only when complexes with cationic liposomes. Aromatic polymer can condense siRNA effectively into negatively charged nanoparticles (NPs) and improve the stability of siRNA. With the motivation of previous research work (Paik et al, development of biocompatible and

immune-stimulatory (pLME) and NAPA separate nanoparticles), we develop the p(LME-co-NAPA) particles for effective siRNA delivery.

1.2. LITERATURE REVIEW

1.2.1. **Polymer and hydrogel**.

Polymers are naturally occurring or synthesized macromolecules that are composed of repeating units and play an essential and ubiquitous role in biological systems. Among the various kinds of polymers such as poly(amino acid) have properties that could mimic protein, good biocompatibility and biodegradability, and feasibility to modulate mechanical properties etc. making it the advantageous choice in drug delivery and in tissue engineering [12, 13]. Hydrophilic Polymer gets crossed linked and from the three-dimensional network in some fashion to produce an elastic structure with a large water-holding capacity it termed the hydrogel. Due to the modulation of material in such a way so that it can embed biologically active agents and can release it in a controlled manner, a hydrogel is considered an attractive material in therapeutic applications.

1.2.1.1. **Poly (amino acid) and Synthesis Strategies' for Amino Acid based functional polymer.** Poly(amino acid) such as poly(aspartic acid), poly(arginine), poly(lysine) and poly-γ—(glutamic acid) are composed of amino acid linked through the amide bond are the naturally occurring polymers. While, these amino acids linked by nonamide bonds, these polymers referred as the pseudo poly(amino acid) which yield the block, branched, hyperbranched or dendron-like architecture and improve the mechanical characteristics and other properties.[13] Further, to yield the amino acid based polymers, different type of strategies were used depending on the functional groups as shown in Figure 1.2.1

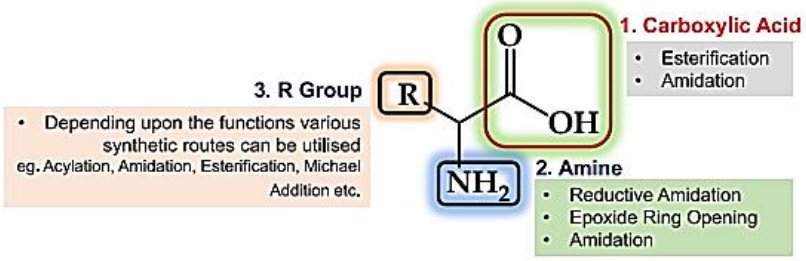


Figure 1.1. 1 *Synthetic strategies of various amino acid functional polymer [14]*

There are different techniques for the synthesis of poly(amino acid) based polymers reported, such as polycondensation (PC), solution or solvent-free thermally activated polycondensation [15], ring-opening polymerization (ROP) [16], reversible addition fragmentation chain-transfer (RAFT) polymerization [17] etc. Direct PC yields optically active functional polymers with a wide poly dispersity index (PDI) [18], however, harsh reactions cleave the labile functional groups, and amino acids lose their zwitterionin in nature. Aside from enduring the zwitterionic nature's increased complexity, water solubility, stimuli-responsive qualities, chiral recognition, and capacity for self-assembly, the addition of amino acids as the side chain employed several advantages. By regulating the circumstances under which the polymers self-assemble, a greater variety of polymeric nanostructures, including vesicles, micelles, nanogels, nanorods, nanotubes, and nanofibers [20], can be created. To yield the side chain pendent structure post modification technique can be used as well as different type of controlled polymerization techniques have been employed, as example ato transfer radical polymerization (ATRP) [19] , nitric oxide-mediated polymerization (NMP) *[20]*, RAFT, living cationic polymerization[21] living polymerization [22], ring-opening polymerization (ROP) method (*[16]*), ring opening metathesis polymerization (ROMP) [23], and metal catalyzed metathesis or insertion polymerization technique [24].

1.2.1.2.**Applications of polymer depend on its physical, chemical, and other properties.** Most studied polymers are commonly employed in the development of functionalized nanotherapeutics due to their diversity. Polymeric nanosystems enhance the therapeutic efficacy by enhancing the stability and half-life of the drug, by improving the biodegradability and by incorporating of smart response.[25] Understanding of particle size, morphology, and processing technique are the useful tool in development of ideal nanosystem[26] based on the architecture of polymeric nanoparticle characterized in different types like nanosphere, nanocapsules, micelles, Polyplex, nanogel and dendrimers[27] The presence of oil in the nanocapsules leads to a vesicular structure[28] while the absence of oil polymeric chain get organized in such way it gives the nanospheres shape. Nano spherical particles are widely used in drug delivery[29] . Another nanotherapeutic delivery system is a dendrimer considered as emerging ideal drug delivery system due to its three-dimensional branched globular structure, mono-dispersity, high symmetricity, surface polyvalency and offer high loading capacity and could be promising in gene delivery[30, 31]. Although dendrimer offers improve targeting and cellular uptake, it also relates to some primary concern about the size limitation, closely related to their toxicity and bio distribution [31]. When the amphiphilic polymer self-aggregates [32, 33] at a critical micellar concentration and forms the internal lipophilic core and external hydrophilic shell, it is termed as the micelles. The micelles are not only of spherical shape but it offers the different shape like, elongated micelles which possess the unique viscoelastic and rheological behavior compared to spherical micelles, and vesicular structure offer the fluidity to particles. Recently, multi compartment type structure in elongated micelles are incorporated by formation of triblock polymer and through the kinetic manipulation. This kind of structure offers different kind of payload with different compartments. Different type of particles, shape

and aspect ratio influence the cellular uptake, pharmacokinetic and pharmacodynamics. The size of particles affects not only the flow rate but also the adhesion process, interaction with biological cells, and uptake mechanism. For example, particles smaller than 5 nm are filtered out of the kidney, particles between 10 and 20 nm can cross the blood-brain barrier and have imaging potential, particles between 20 and 100 nm can easily escape physiological barriers and have a high potential for circulation, particles between 200 and 1 µm are generally cleared by the spleen [34]. Among the various shapes of the nanoparticle, spherical shape of particle is widely studied and asymmetrical shaped particles considered as an advantageous in various applications like in tissue engineering, immunoesnsing etc. For an example irregular shaped poly(methyl vinyl ether-*co*-maleic anhydride) and various lipids majorly accumulated in spleen while sphere shaped in liver[35]. Similarly, disk-shaped particles with a longer blood circulation period and a greater pulmonary anti-ICAM to IgG ISI were found in comparison to spherical ICAM-1-coated polystyrene. [36]. In tissue engineering and in regenerative medicine[37, 38], nanotopograpy plays important role like human pluripotent stem cells sense the topographic arrangement and relay on biophysical signal and regulates the signaling network followed with hPSCs differentiation to specific type of neuron.[39]

The creation of smart stimuli-responsive polymeric vesicles, micelles, and dendrimers for anticancer therapy are the main drivers of recent advancements in polymeric nanotherapeutics.[40] Amino acid based polymer majorly considered as the valuable tool in development of pH responsive and thermo responsive polymer and drown the height attention due to its biocompatibility, water solubility and formation of heirachical structure [41, 42]. Anionic and cationic polymers are two categories of pH-responsive polymers. [43-45]. Polyacids, such as PAA, PMA and poly(sulphonamides) are often used as anionic pH-

responsive polymers [46, 47]. poly(dimethylaminoethyl methacrylate) (PDMAEMA), poly(2-(diisopropylamino)ethyl methacrylate, Poly(ethyleneimine) (PEI) and poly(4-vinyl pyridine) (PVP) are examples of polybases that are cationic polymers [47-49]. The presence of NH_2 /COOH functionalities in polyamino acid-based polymers [50, 51] get involved in protonation or deprotonation in response to changing the pH of a solution. Three-dimensional cross-linked polymeric networks with ionic pendant groups can ionize and produce fixed charges at certain pH and ionic strength. The pH-dependent swelling and de-swelling is caused by electrostatic repulsive force. [52, 53]. The most often researched pH-responsive polymers for gel synthesis are PMA, PAA and PDMAEMA. These polymers' gels have been used in tissue engineering, drug delivery, artificial mussels, and protein recycling.[43] pH-responsive polymer not only offers the pH based swelling or deswelling, but it is also helpful in the development of endosomal escaping polymer [54, 55] Since PEI, poly(amino ester), poly(L-lysine), poly(2-(dimethylamino)ethyl methacrylate) and polyamidoamine] can promote osmotic enlargement of endosomes, ultimately resulting in endosomal rupture, they can buffer the endosomal acidification by protonation [56-58]. While the hydrophobic amino acids enhance the interaction of linear to branched chain polymer with lipid membranes [59]. Because of their redox activity and permeability to cell membranes, researchers have recently concentrated on developing redox responsive polymers that can disrupt the intracellular redox state. Notably, to suppress the viability of the cancer cells selectively [60]. As example, hyperbranched polyglycerol (HPG) with hydrophilic and biocompatible nature was attached onto the hydrophobic anticancer medication 7-ethyl-10-hydroxy-camptothecin (SN38) via an H_2O_2-responsive thio-ether linkage, allowing the drug to encapsulate cinnamaldehyde (CA). CA-loaded HPG-2S-SN38

nano micelles can enter easily into the cancer cells and can release CA and SN38 due to the intracellular oxidative environment [61].

1.2.1.3. **Amino acid-based Polymers and their biomedical applications.** Naturally occurring precursors of amino acids are expected to induce biocompatibility, which makes them an intriguing candidate for a variety of biomedical applications, including drug delivery, gene delivery, cell delivery, scaffold preparation, the formation of polymer-antibody bioconjugates, and the development of polymer prodrugs. For example, Poly (γ-glutamic acid)(PLGA) is a naturally occurring biocompatible and non-immunogenic poly-peptide polymer and is widely used in various medical applications [62]. PLGA in conjugation with different moieties exhibits pH responsiveness,[63] and shows excellent adhesive strength in visceral tissue[64], and its composite inherited good mechanical strength with self-healing ability, chondrogenic differentiation and cutaneous wound healing efficiency [65]. Electrostatic interaction between cationic polymers and negatively charged nucleic acids form the new class of polymer referred to as polyplexes. polyplexes have garnered significant attention due to their ability to encapsulate and protect genetic material [66], navigate cellular barriers [67], and orchestrate the controlled release of therapeutic cargo[68]. Similarly, dendrimers are also a valuable tool for the delivery of genetic material due to high branches consisting of high-function moiety which enhances the interaction with genetic material referred as the dendriplexes. Intricacies of polyplex/ dendriplexes interactions with cell membranes and exploit endocytic pathways for efficient internalization. [6] Along with gene delivery, cancer treatment, amino acid based polymer also offers structure based antibacterial activity[69]. For antibacterial/antimicrobial applications[70] it is important to optimize the hydrophilic/hydrophobic balance is a crucial

parameter, as with an increase in amino acid side-chain hydrophobicity, modulation of alkyl groups of different chain lengths offers the increase in antibacterial activity and toxicity is observed. [71] Fmoc-methionine (Fmoc-M), Fmoc-tryptophan (Fmoc-W) and Fmoc-tyrosine (Fmoc-Y) based hydrogel show the antibacterial properties. While formation of nano fiber compare to hydrogel, the higher level of antibacterial property has been observed.[72] Similar to this, the pendant groups of lysine and arginine-based moieties in poly ester amide micelle nanocarriers have an intrinsic antibacterial capability, and this killing ability can be increased by grafting the medication. [73] These antibacterial and antifungal properties are the major requirement in development of scaffold[74] and regenerative cell delivery vehicle.[75]. As enriched medium with growth factor and supplement facilitate the growth of other infectious organism and hinder the growth of the healing cells and increases the immune inflammatory signaling, ultimately hindered healing capacity.[76] Antibody-biopolymer conjugation shows the add-on to old immunization method and widely exploring towards the development of immunotherapy especially in case of cancer therapy[77], in autoimmune diseases and in diagnostic application. Polymer conjugation with antibody/antigen, increases the protein stability, reduce the aggregation, multimerization for increase assay sensitivity or signal detection through phase separation.[78] However, use of polymer offers several advantage but still faces the many challenges. Like development of prodrug[79] and polymer−drug conjugates[80]; they often experience two problems i.e., complicated to design biodegradable linker which could be easily get cleaved by metabolic process and in the development requirement of multistep reactions like protection and deprotection approaches. Which further limit the large scale

production of polymer prodrug and conjugates. Hence development of functional polymer is always a need[81].

1.2.2. **Advantages of Computational methodologies for biomedical Applications.**

In recent time, the design and development of novel delivery system provides the efficient therapeutic approach.[82, 83] And use of the computational approach reduces the burden of time, cost and unpredictable error which are associated with the traditional trial and error experiments.[83] It addresses the broad range of question, like give the prediction of self-assembly, resulting aggregates and colloidal stability, drug loading capacity and mechanism, interaction with biomolecules like serum protein, miRNA/siRNA, heparin etc.,[84] and effect on of environmental condition like pH, temperature and external stimulus.[85]

Computer simulation and modellings of materials and processing with algorithms generate the statistically valid conclusions for the output.[86] In material science, Quantum mechanical calculations[87] were explored to elucidate the optical, magnetic, electrical and mechanical properties of CNTs, magnetic NPs, quantum dots (QDs) and similar system.[85] The resulting method, known as Molecular Dynamics (MD), has extensive application in soft-condensed materials and biological systems.[88, 89] Utilizing the software programs AMBER, CHARMM, LAMPPS, GROMACS, and NAMD. This method's main drawbacks are its time-consuming computation and its failure to account for certain aspects, such as point charges, which can have an impact on charge transfer, polarization, and other electronic effects that could be crucial for carbon nanotubes.[90]

Furthermore, introduction of density functional theory (DFT) [91] has tremendously aided valuable asset in development of drug delivery material.[92, 93] DFT provides exceptional levels of accuracy and is less expensive in terms of processing resources. DFT calculation

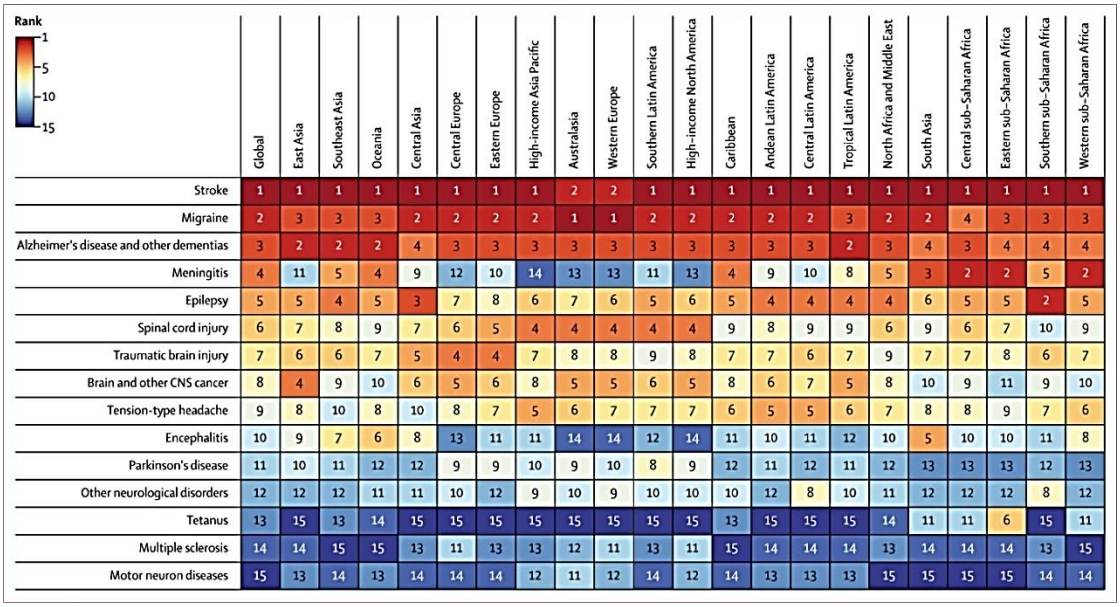
gives the fundamental information about geometric structure, electrical and optical properties, thermodynamic properties including energy. Energy, geometric structure, electrical, and optical properties are among the fundamental information that computations are utilized to solve primarily in DFT calculations. In terms of material design, it provides valuable theoretical guidance and predictions. Schrödinger equation. $H\psi = E\psi$ Where ψ is wave function, H is Hamiltonian and E is the system's energy. In case, Despite of various basis set, for polymer, organic drugs the wide used function and basis set is B3LYP and 6-31G(d, p).[94] Similarly, in computational drug discovery, molecular docking[95] [96] is an important tool. This approach mainly used to model interaction between small molecules and protein to evaluate the behavior of the small molecule at the binding site of the target protein, changes in the orientation and the assessment of binding affinity.[97]

1.2.3. **Neuron regenerative approaches.** Neurodegeneration is a process which leads to irreversible damage to nervous system caused by multifactorial genetic, environmental and endogenous factors. While regrowth of the nervous tissue[98] via external stimuli which leads the generation of new neuron, axon, synapses and glial cells is a neuroregeneration[99-101]. Neuron, are electrically excitable cell, involve in transmission of information. The nervous system is a complex regulatory network comprised of CNS and PNS. CNS is composed of two main cell type neurons and neuroglial cells consist of astrocytes, microglial and oligodendrocytes while in PNS Schwann cells are the glial cell type involve regenerative mechanism. In PNS, Schwann cells promote peripheral nerve regeneration by clearing myelin debris in injury and synthesizing myelin sheath, in response to Wallerian degeneration. Endogenous electric field facilitates the Schwann cell accumulation to longitudinally newly formed Nerve Bridge and regulates signaling pathway by releasing

NGF, BDNF, etc. and guides axon regeneration. NGF overexpression selectively recovers sensory axons while BDNF and GDNF impair motor functional recovery[102]. Neurons communicate via environmental changes and body response activities while Glia supports neighboring neurons for homeostasis maintenance [103]. CNS possess lack of regenerative capacity compared to the PNS due to the existence of hostile microenvironment, hence neutralization of the microenvironment helps in regeneration. This research area is a current thrust area of research in tissue engineering and in pharmaceuticals due lack of availability of novel and effective regenerative medicine [104]. Structural, biochemical and electrical abnormalities in the brain, spinal cord and nerves are the cause of injuries that results a range of neurological disorders and which is a worldwide second leading cause of death.

1.2.3.1. **Global Statistic of Neuro degeneration and Cerebral Cancer.** Neurological burden measure in terms of DALYs (disability-adjusted life years) which is continuously increasing .[105] As per WHO report in 2019, 55million people affected by neurological disorder and by 2050 this number may increase to 139 million people. In India the prevalence rates ranged from 967-4,070 with a mean of 2394 per 100,000 populations excluding neuroinfections and traumatic injuries.[106] 50 million people globally are suffering from epilepsy and 24 million from Alzheimer and other dementias. People from all over the world are suffering from Neurological disorders. It is also reported that, traumatic brain injury (TBI) is also a leading cause of death and disability among in United States (Ref. Report of Congress: Traumatic Brain Injury in the United States)). The proportional contribution of neurological diseases and geographical distribution is represented in the figure (1.1.2). Similarly, another major contributor is brain and CNS cancer. As per Global Cancer Statistics

2020, age-standardized incidence rates of CNS cancer increased globally by 17•3% and the incident cases were highest in China, the USA, and India.[107]



Rank	Global	East Asia	Southeast Asia	Oceania	Central Asia	Central Europe	Eastern Europe	High-income Asia Pacific	Australasia	Western Europe	Southern Latin America	High-income North America	Caribbean	Andean Latin America	Central Latin America	Tropical Latin America	North Africa and Middle East	South Asia	Central sub-Saharan Africa	Eastern sub-Saharan Africa	Southern sub-Saharan Africa	Western sub-Saharan Africa
Stroke	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Migraine	2	3	3	3	2	2	2	2	1	1	2	2	2	2	2	3	2	2	4	3	3	3
Alzheimer's disease and other dementias	3	2	2	2	4	3	3	3	3	3	3	3	3	3	3	2	3	4	3	4	4	4
Meningitis	4	11	5	4	9	12	10	14	13	13	11	13	4	9	10	8	5	3	2	2	5	2
Epilepsy	5	5	4	5	3	7	8	6	7	6	5	6	5	4	4	4	4	6	5	5	2	5
Spinal cord injury	6	7	8	9	7	6	5	4	4	4	4	4	9	8	9	9	6	9	6	7	10	9
Traumatic brain injury	7	6	6	7	5	4	4	7	8	8	9	8	7	7	6	7	9	7	7	8	6	7
Brain and other CNS cancer	8	4	9	10	6	5	6	8	5	5	6	5	8	6	7	5	8	10	9	11	9	10
Tension-type headache	9	8	10	8	10	8	7	5	6	7	7	7	6	5	5	6	7	8	8	9	7	6
Encephalitis	10	9	7	6	8	13	11	11	14	14	12	14	11	10	11	12	10	5	10	10	11	8
Parkinson's disease	11	10	11	12	12	9	9	10	9	10	8	9	12	11	12	11	12	13	13	13	12	13
Other neurological disorders	12	12	12	11	11	10	12	9	10	9	10	10	10	12	8	10	11	12	12	12	8	12
Tetanus	13	15	13	14	15	15	15	15	15	15	15	15	13	15	15	15	14	11	11	6	15	11
Multiple sclerosis	14	14	15	15	13	11	13	13	12	11	13	11	15	14	14	14	13	14	14	14	13	15
Motor neuron diseases	15	13	14	13	14	14	14	12	11	12	14	12	14	13	13	13	15	15	15	15	14	14

Figure 1.1. 2 *Age-standardised DALY rates ranking for different neurological disorders reported by GBD(Year 2015)*

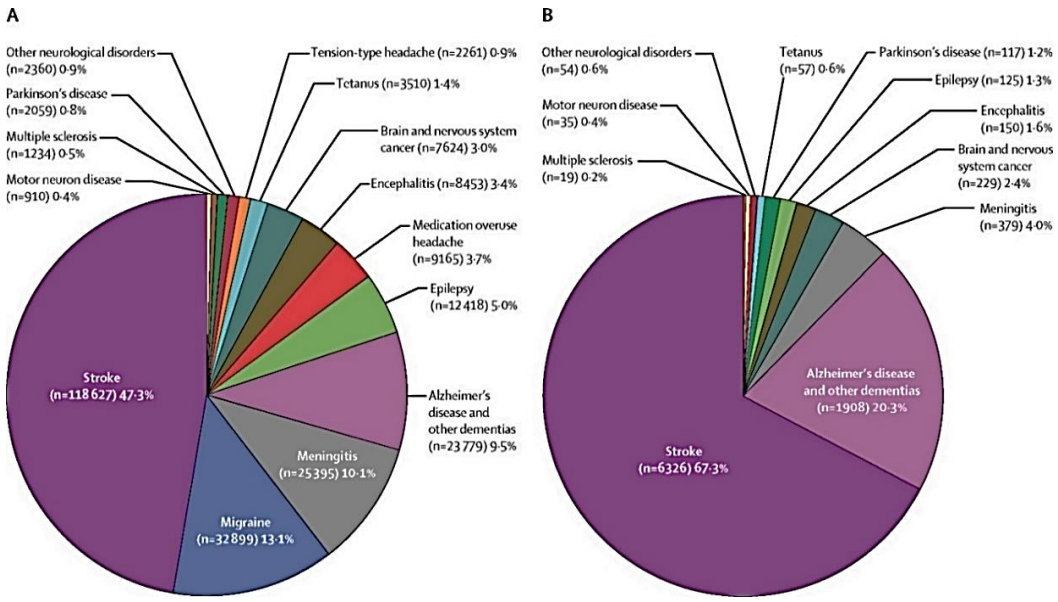


Figure 1.1. 3 *Various neurological disorders in 2015*

1.2.3.2. **Vulnerability of adult neuron for degeneration.** In the brain, the frontal lobe is the most vulnerable area for damage to traumatic brain injuries (TBI).[108] In terms of stress, the prefrontal cortex is the most sensitive area for stress-related effects and is highly important for cognitive disabilities.[109] This prefrontal cortex and hippocampus interconnection plays important role in the assimilation of new memories into pre-existing networks of knowledge and memory consolidation.[110] Chronic stress majorly accelerates hippocampal damage.[111] It is because excessive local neuronal activity increases amyloid deposition and hub regions possess the highest level of activity and that hub vulnerability[112]. Similarly, in generalized mechanism, adrenal glucocorticoids activate glucocorticoid receptors [113] followed by the release of glutamate leads to activation of glutamate receptors in normal conditions. In aging, the molecular and cellular events change and lead to the excessive activation of glutamate receptors[114]. This excessive activation of glutamate receptors damages the dendrites, increased Ca^{2+} influx and ROS production leads to damage to dendrites and cell death. Hence the dendrites compromise energy metabolism and make neurons more vulnerable to glutamate excitotoxicity.[108] Similar to this, certain mitochondrial abnormalities are more susceptible to certain cell types due to certain causes. Together with the mitochondrial single-stranded DNA binding protein (mtSSB) and the replicative mtDNA helicase TWINKLE, the mitochondrial replisome is able to continuously replicate both mtDNA strands [118]. And multiple mutations during this process is majorly responsible for neuromuscular defects, since a unique susceptibility of cerebellar Purkinje cells to modifications in mtDNA as a result of compromised replication is noted [115-117]. Dopamine metabolism is linked to the generation of several intermediate metabolites,

reactive oxygen species (ROS), and a build-up of mtDNA deletions that cause pathological states like AD and PD [118].

1.2.3.3. **Role of angiogenesis in neuron growth and neurotherapy.** Formation of new functional neuron from neuronal progenitor cells are referred as neurogenesis while formation of new micro vessels from pre-existing branching vessels is referred as angiogenesis.[119-121] In development CNS, both the processes are the coupled process.[122] Existing treatment approaches for neuro-therapy.[123] considered as failed therapy due to lack of consideration in development of cerebral microvasculature. In most of CNS pathophysiological conditions, ischemic stroke, vascular Parkisonism, amyotrophic lateral sclerosis, Alzheimers disease, vascular dementia, and aging, reduction in vessel density and cerebral blood flow occurs which leads the loss in cognitive function.[124] It is important to consider that Neuronal progenitor cell (NPCs) present in close proximity with blood vessels which provides the guidance to NPCs[125] for neurogenesis and hence development of cerebral microvasculature play important role in recovery of adult neurogenesis.[126] Similarly, growth factors play wide role in accelerated nerve regeneration.[127] Neurotrophin nerve growth factor (NGF) promotes the neuron survival and differentiation [128], while Fibroblast growth factor 2 and vascular endothelial growth factor are pro-angiogenic factors.[129] FGF and VEGF promote brain cell proliferation and are crucial in the development of neurodegenerative illnesses of the peripheral nervous system, whereas NGF simultaneously induces angiogenesis and arteriogenesis, speeding up hemodynamic recovery. [130, 131] And hence, polymeric material need to be pro-angiogenic (pro-angiogenic) and growth factor stimulating material [132-135] to improve the regenerative properties.

1.2.3.4. **Current status Polymer used in Neuron repair and growth.** In neuroregenerative area most of the research are focused towards the prevention of neuronal damage [136, 137],

2. Table 1 Natural polymers used in neural tissue engineering, [138]

Natural Polymer	**Biocompatibility *in vitro***	**Biocompatibility *in vivo***	**Application**
Pre-clinical Studies			
Collagen	DRGs	Non-human primates, Rats, Dogs, Cats, Mice	Nerve guide, Hydrogel Scaffold, Nerve conduits, entubulation
Gelatin	C17.2, PC12, hC-MSCs, RT4-D6P2T, Allogenic RMCs, Primary RSCs		Electrospun conduits, Nerve Conducts for Schwann cells, Scaffold, Bioink
Elastin	PC12	Mice, Rats	Thermally Responsive ELPs, ELPs Intranasal Drug Delivery
Hyaluronic Acid	NPCs from forebrain cortical neuroepithelium of E13.5 rats, C17.2 cells, ReNcellsNPCs, , GRPs, NSCs, RSC96, PC12	Rats Rabbits, Rats	Hydrogel Hydrogel, Nerve Conduit, Drug delivery, Coating
Alginate	PDLMSCs and GMSCs, PC12, NSCs	Cats, Rats	Gel, Spong, Hybrid scaffold
Chitosan	PC12, NPCs from forebrain cortical neuroepithelium of C57 fetal rats, Neuro-2a, Schwann cells from Sprague-Dawley rats, BMSCs, U373, GFP+RG3.6, hNSC	Dogs, Rats, Mice	Hydrogel, Micro/nano vehicle, Scaffold
Keratin	RT4-D6P2T, Glial cells	Mice, Rabbits, primates	Nerve guide, hydrogel, Nanofibrous scaffold
Silk	hippocampal neurons, DRGs,NSCs, Primary NT2, PC12, NSCs	Rats and Dogs	Hydrogel, Electrospun scaffold
Clinical Studies			
Collagen		Reconstruction of Peripheral nerve	NeuraGen®
		Reconstruction of Peripheral nerve	Neuromaix®

Limited research are directed towards the functional recovery of injured neuron. In the current research strategies, polymer scaffolds, hydrogel nanoparticles and nerve conducts are aimed to use in treatment of CNS and PNS [139, 140]. Among the various kind of polymeric material, only collagen polymer conduits have been successfully tested in clinical trials for peripheral nerve regeneration [138]. The natural polymers which are explored for neural tissue engineering have been listed below in Table 1. Similarly, Synthetic amino acid-based polymers have been explored for their potential applications in neurogenesis and angiogenesis due to their tunable properties and versatility. This polymer offers the several advantages like tailorable properties, biocompatibility, controlled degradation, functionalization for bioactivity, reduce the risk of disease transmission etc. However, its uses limited due to limited mimicry to native tissue [141], immunogenisity [142, 143], toxicity of by-product, lack of inherent bioactivity, and complex procedure along with high cost. The presently explored polymer in neural tissue repair and in angiogenesis are listed in Table 2

Table 2 *Polymer used for the angiogenesis and neurogenesis*

	Polymer	Conjugate/ growth factor for angiogenesis	Neurogenesis	Angiogenesis	Other Comment	Reference
Natural Polymer	hyaluronic acid (HA) hydrogel	VEGF mimetic peptide (KLTWQELYQLKYKGI		yes	inhibit the formation of glial scars	[144]
Synthetic Polymer	poly (urethane amino sulfa-methazine) (PUASM)	Stromal cell-derived factor-1α	yes	yes	-	[145]
	Tetramethylpyrazine (TMP)	-		yes	TMP encourages nerve regeneration and the healing of nerve abnormalities by down-regulating miR-497-5p to target EGFL7.	[146]

	poly(ethylene glycol)-ELR IKVAV-containing hydrogels	laminin-derived pentapeptide (IKVAV) and VEGF-mimetic peptide (QK)	yes	yes	spatiotemporally controlled angiogenesis and neurogenesis *in vivo*	[147]
Amino Acid based polymers	Poly-Lactic Acid (PLA)		yes	yes	Degradation induce lactate involved in progenitor maintenance	[148]
	Poly(Lactic-co-Glycolic Acid)	VEGF miRNA-129-5p; basic fibroblast growth factor (bFGF); erythropoietin	yes	yes	Neuroprotection; microglia activation; peripheral nerve regeneration; Sensory motor function	[149] [150] [151]
	piezoelectric WH and poly(ε-capro-lactone) Scaffold	Mg+	yes	yes	neuro-vascularized bone tissue	[152]
	poly(aspart-amide) hydrogel	dopamine	yes		Using SH-SY5Y neuroblastoma cell line	
	Poly-glutamic acid dopped polypyrrole	surface was modified with polylysine and laminin	yes		Micro-patterned guidance for neuritis extension	[153]
	alginate/poly(γ-glutamic acid) scaffolds	TATVHL peptide	yes		iPS cells	[154]
	Heparinized chitosan/poly(γ-glutamic acid) nanoparticles	fibroblast growth factor (bFGF)		yes	-	[155]

1.2.4. Basic information on Cancer. Cancer is malfunction in cell division of body cells of a human body. It can generate in any part of body and multiply uncontrollably to form trillions of cells. I normal process cells age and die with replacement of new cells through process of cell division. Sometimes malfunction in this process occurs and some cells escape from this orderly process and gradually they form tumor body in case of solid tumors sometimes benign (non-cancerous) and occasionally malignant (cancerous). Malignant tumor cells escape from confined space and travel to new places and tissue forming multiple tumors in multiple locations through process of metastasis. Benign tumors

do not spread to other parts of body and often removed with successful treatment. However, malignant tumors spread and undergo metastasis with various degree from slow metastasis to highly invading tumors like Triple negative breast cancer.

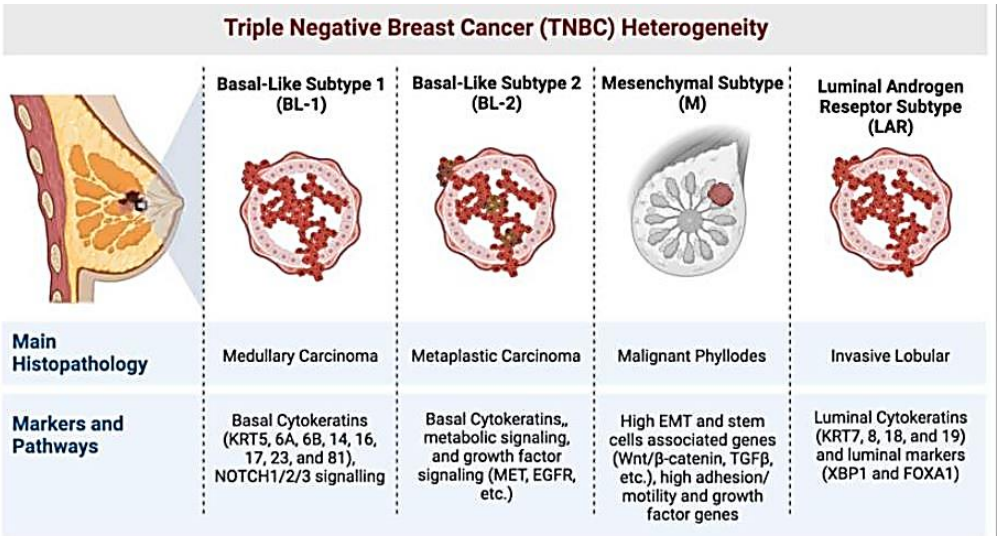


Figure 1.1. 4 *Schematic of TNBC heterogeneity showing TNBC subtype's main histopathology, markers, and signaling pathways [156]*

1.2.4.1. G**lobal Statistics of Cancer.** According to the World health organization, 2020 report, it is estimated that cancer is the second or first leading cause of death, accounting for nearly one in six deaths. Overall the incidence rate and the mortality rate are rapidly growing worldwide. For both sexes, the top diagnosed cancers are breast cancer (11.7%), prostate cancer (7.3%), colorectal cancer (10.0%), Lung cancer (11.4%), and stomach cancer (5.6%) (Figure).[157] The females in all age groups, the estimated newer incidence mean/estimated mortality are breast cancer cervical cancer (16.5/16.2%), (27.7%/23.5%), and ovarian cancer (6.2/6.5%). Triple-negative cancer is categorized in subgroups based on heterogeneity, and lack of receptor expression and it constitutes 12-24% of all breast cancer.[158] Only in India, the prevalence is estimated to be 25.04% (95% CI, 23.42% to 26.67%) and the average survival rate from disease using current therapy is ~10.2 months. Similarly, glioblastoma and neuroblastoma is other aggressive types of cancer.

Glioblastomas are the deadliest brain tumors, but neuroblastomas are the most common extracranial cancers in children. Glioblastoma has an incidence of 3.21 per 100,000 populations in the median age population, with a poor rate of survival approximated 40% for 1 year and 17% for 2 years. Hence there is an urgent need for newer therapeutics, especially against the aggressive type of cancer.[159]

1.2.4.2. Genetic alteration and Global challenge in treatment of Aggressive cancer.

Conventional therapies create the lot of elevated side effects and hence oncological research putting lots of efforts towards the finding newer therapies using nanomedicine approach. Aggressiveness, high heterogeneity, invasive characteristics and absence of therapeutic targets are major challenge in treatment of aggressive cancer treatment such as treatment of TNBC and glioblastoma. Heterogeneity is an indirect consequence of altered microenvironment of tumor where, increased glycolysis decreases the pH which leads the acid-mediated invasion of cancer cell in normal tissue. Adaptation to acidosis make the cancer cells more proliferative, migratory and increased in expression of vasculature marker.[160] Cancer in Adolescent young adults (AYA) posited the unique cancer biology, often harbor unfavorable genetic changes that portend a worse prognosis and are often positive for TNBC with high relapse rate. In the AYA population, breast cancer is inherently more aggressive due to BRCA1 mutation.[161] Still, the nature of cancer stem cells remains unclear.[162] Lehmann et al. identified six TNBC subtypes—two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype—based on gene ontology and differential gene expression analysis. They also linked these subtypes to various mutations, such as aberrant PTEN expression, five distinct microRNA aberrations, high MYC

expression, TP53 mutation, RB1 loss, and WNT signaling. And the poor prognosis and poor clinical result is connected with AKT1 copy gain/high mRNA expression in the BL-2, PTEN-low/miRNA-low high RhoA signaling in the BL-1 tumors, and high programmed cell death 1 (PD1) expression in IM [162]. Furthermore, as Microarray expression analysis revealed that in glioblastoma high expression of GFAP, VEGFA, and genes involved in cell survival, migration and cell cycle pathways are overexpressed.[156].

1.2.4.3. Amino acid transporters and the therapeutic target in Cancer. In Cancer, uptake of amino acid and metabolism aberrantly upregulated to facilitate the cancer cell survival and proliferation under genotoxic, oxidative, and nutritional stress condition.[163] However, the cancer cells are unable to synthesize essential/non-essential amino acid, hence the amino acid transporters are overexpressed in cancer cells. There are many amino acid transporter which are unregulated human among them four main transporters SLC1A5, SLC7A5, SLC7A11, and SLC6A14 having the capability to promote cancer growth and chemo-resistance.[164] these transporter expression varies in different type of cancer such as in breast cancer SCL7A5 is highly expressed. SLC7A5 is a system L(leucine preferring) amino acid transporter, which is an antiporter that exports the intracellular glutamine to facilitate the uptake of substrate leucine to allow leucine dependent cell proliferation.[165] This transporter has the high affinity for branched chain amino acid such as leucine, phenylalanine, and tryptophan etc. the expression of SLC7A5 is regulated by HIF2α and therefore, it is highly expressed cancer cells as in cancer hypoxia is observed majorly.[164] On the other hand SLC1A5 facilitates the glutamate transport in cancer cells. Further, Extracellular cystine is transported by SLC7A11. Another name for SLC6A14 is ATB0,+ (amino acid transporter with cationic, indicated by "+," and neutral, indicated by

"0," amino acids as substrates). Numerous cancers, including pancreatic, colon, cervical, and estrogen receptor-positive breast cancers, have it overexpressed. Gemcitabine-resistant pancreatic cancer cells overexpress the neutral amino acid transporter SLC7A8, which is Na+-independent. Therefore, targeting to amino acid transporter through the blocker or designing the delivery system which facilitates the delivery of drug through these transporters could become valuable choice in anticancer therapeutics.

1.2.4.4. **Role of polymers in cancer treatment.** Unique feature of pharmacokinetic, circulation time, biodegradability along with biocompatibility make the polymer as an optimal tool in drug delivery. Now a day's smart biopolymer are more applicable and fascinating in the biomedical applications. Modulation polymer in nanomedicine minimizes its side effects and employ the effective drug delivery system. Now a day, use of drug free macromolecular therapeutics are considered as the novel class of therapeutics. The concept was originally introduced by Kopecek and co-worker in 2010. Where coiled coil peptide interacted with B-cell lymphomas highly expressing CD20 receptor in absence of low molecular weight drug. Similarly, Sachharides based polymer also shows the feature of biorecognition. Anionic polymers of the polymethacrylate class have the ability to have pharmacological effects and are linked to immune system activation. [166]. Although the concept for biorecognition of polymer introduce in few years back but only few polymer are known with this concept. Zhong and co-worker introduced the biodegradable tri-block co-polymer based on guanidinium-functionalized polycarbonate which is effective in killing a broad spectrum of drug resistance cancer.[167] Similarly, Takahashi and co-worker introduced polymer which target the anionic lipid exposed on cancer and effective in prostate cancer and especially against dormant cancer cells.[168] we considered that

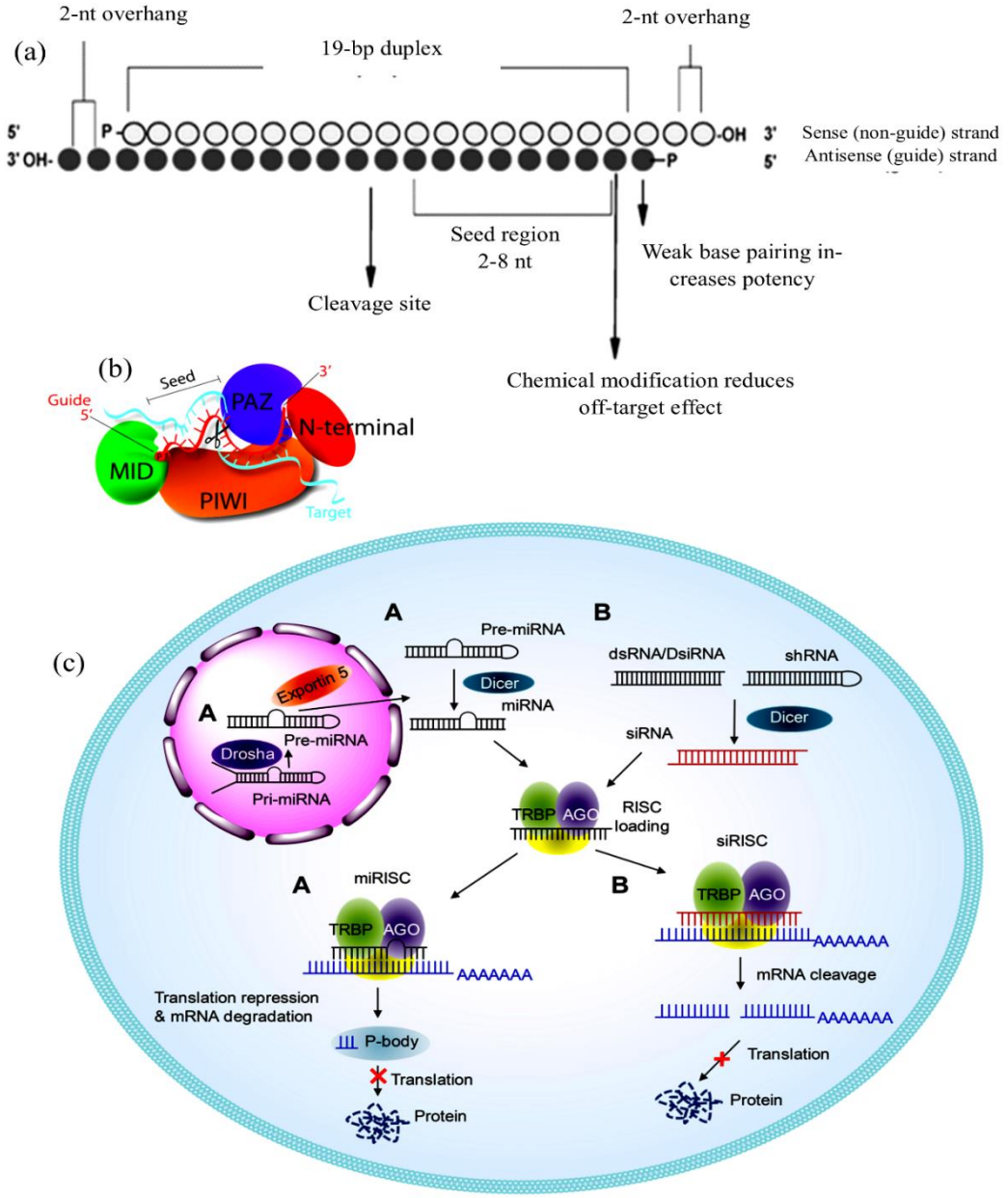
there is a huge scope in development of anti-angiogenic anti-cancer polymer which is unknown to the best of our knowledge.

1.2.4.5. **Role of siRNA for cancer treatment.** siRNA therapeutics have been widely accepted in the research fraternity for their applications in cancer and diseases that are undruggable by small molecules and antibody drugs. It has been confirmed that siRNA inhibits the genes and cell proliferation in various mammalian cell lines such as HEK and Hela.[169] Moreover, these therapeutics rescue healthy cells from damage by specifically targeting cancerous cells. One major benefit arises from the co-delivery of siRNA and chemotherapeutic drugs by increasing the silencing and thereby increasing the sensitivity of current chemotherapy resistance.[170] siRNA therapeutics are used in different cancers such as lung, pancreatic, breast, colorectal, ovarian, hepatocellular, gastric, and cervical cancer.[171] Such a wide scope has been an eye-catcher for researchers in drug discovery and development.[172]

1.2.4.6. **siRNA mediated RNA interference mechanism.** The RNAi mechanism is dsRNA mediated potent sequence-specific inhibition. Dicer has region homology to RDE1/ QDE2/ Argonaut family and cleaves the double-stranded RNAs which produces putative guide RNAs and initiate the RNAi mechanism.[173, 174] In human, among the Argonaut 1-4 proteins, Argonaut 2 plays a critical role in RNAi along with miRNA and siRNA. Dicer shared the PAZ domain of the Argonaut and initiates RNAi by processing dsRNA into 22 nucleotide fragment. AGO2/Dicer complex facilitates the incorporation of siRNAs into RISC complexes and Dicer get dissociated.[175] siRNA symmetry and thermodynamic differences in the base-pairing stabilities of the 5’ ends have crucial role in guide strand selection .[176] Protein sensor Trans-activation responsive (TAR) RNA-

binding protein (TRBP) and catalytic counterpart preferentially sense the siRNA asymmetry and highest thermodynamically stable guide strand promotes the loading of guide/ antisense strand into Ago2 protein [177, 178] which activates the RISC Loading complex.[179] Loading leads to the cleavage of sense strand and activates guide strand loaded RISC complex for silencing of target. AGO proteins are composed of bilobal architecture, i.e, amino-terminal PAZ (PIWI/Argonaut/Zwille), C terminal MID (middle) and PIWI (P-element-induced wimpy testes) domains. The phosphorylated 5'-teminal of guide strand recognizes the MID domain with strong binding and multiple contacts to the charged phosphate backbone while 3'terminal hydroxyl group recognizes the PAZ domain while PIWI has the role in epigenetic regulation.[180, 181] There is direct connection between PAZ/3'-overhang binding affinity, siRNA's potency and specificity observed. Modified 3'overhang affects the preferential strand selection and moderates to strong binding between PAZ/3'overhang required for the higher success of the RNAi activity. 3' overhang removal of nucleobase with increase in steric hindrance and entiomeric substitution indicates negative effect, while the modification of the sugar and phosphate provides positive effects on the siRNA potency.[177, 182]

1.2.4.7. **Chemically modified siRNA and its advantages as therapeutics.** Although siRNA therapeutics can treat any gene of interest and require less time for research and development than small molecule therapeutics and antibodies. There exist two major issues: stability and off-targets.[183] Stability is a major issue for any therapeutics when presented intracellularly. siRNA tends to degrade quickly by endonucleases into nucleotide fragments. Similarly, off-targets may cause the knockdown of irrelevant genes mediated by sense or anti-sense strands of the siRNA.[184].



- ***Figure 1.1. 5. siRNA mediated*** *RNA interference mechanism. (a) Structural orientation of siRNA which composed of 19 nucleotide with overhang structure. (b) Argounote 2 protein and its structural domains (c) siRNA or miRNA mediated silencing mechanism where siRNA loaded with TRBP and AGO2 protein and form the active RISC complex, which eventually degrade the mRNA and inhibit the translation [185]*

- Further, it may have systemic effects on blood and lymphatic systems.[186] It has also been found to cause immune-stimulatory responses via cytokine and interferon production.[187] To maneuver towards stable and specific siRNA therapeutics, the

researchers have developed strategies to modify the chemistries of the siRNA molecules. These are not only providing a balance between stability and on-target but also efficiently suppress immune responses with enhanced bioactivity of siRNA inside the cells.[188]

Chemical modifications are made on three sites (Figure): phosphate backbone, ribose sugar moiety, and nitrogenous base. These modifications can be made in any of the combinations which can enhance gene silencing and stability.[189] there have been lots of efforts made in this state-of-the-art technology for acquiring siRNA modification patterns. One such effort was done by preparing a database for experimentally validated chemically modified siRNA in siRNAmod.[190] The modifications are made on both sense and anti-sense strands to mimic RNA structurally and thermodynamically.[191] Hence, there is no accurate pattern for siRNA modifications whether complete or partial modifications are carried out. It also depends on the target gene, tissue or cell of interest, organism, and most importantly the method of transfection to ensure gene silencing.[192]

1.2.4.8.siRNA and Drugs in cancer treatment. siRNA therapeutics has been well known for its specificity by targeting mRNA than conventional drugs which target enzymes, receptors, and ion channels. Moreover, the spectrum of disease targets is also wider than current chemical drugs.[193] Cancer is one of the major diseases at this point of time in human history. The available therapeutics are very painful and costly. RNAi therapeutics are seen to overcome such challenges in clinical settings by targeting all the possible oncogenes. However, the list of FDA-approved drugs is only six in number till today (Table), of which none for cancer-related diseases.[194] Although it is very far from targeting cancer clinically, one could find numerous examples in vitro and in vivo to treat various cancers.[195] The reports of efficient therapeutic delivery and occurrence of adverse effects at present time hinder the development

of siRNA-based cancer therapy.[196] Given the hurdles presented by various factors such as poor cell uptake, endocytosis, attack by endonucleases, and poor bioavailability in target cell types. Nano-formulated siRNA-based therapeutics approach has been explored extensively. Such nanocarriers could be lipids, micelles, peptides, polymers, dendrimers, carbohydrates, metals, CNTs, and a combination of all of these which increase cancer therapeutics efficacy and safety profile.[197]

1.2.4.9.**Advantages of siRNA/ Drug for co-delivery.** Cancer therapeutics have been the story of increasing the efficacy of treatment and reducing the complications post-treatment. Researchers continually pitched for the co-delivery of the therapeutic drugs which proved clinically effective. [198] The major merits of co-delivery. include synergistic effect, reduced toxicity, and overcoming multidrug resistance (MDR).

Table 3 *Clinically approved siRNA therapeutics*

Active ingrediants	Approval	Indication and usage	Target organ	Target gene	References
Nedosiran	2023	Reduced levels of oxalate in the urine in patients with primary hyperoxaluria type 1 and largely intact kidneys	Liver	lactate dehydrogenase A (LDHA)	[199]
Vutrisiran	2022	treat the genetic transthyretin-mediated amyloidosis polyneuropathy.	Liver	transthyretin (TTR)	[200]
Inclisiran	2021	As an adjunctive treatment for clinical atherosclerotic cardiovascular disease or heterozygous familial hypercholesterolemia	Liver	proprotein convertase subtilisin/kexin type 9 (PCSK9)	[201]
Lumasiran	2020	treat hyperoxaluria type 1	Liver	hydroxy acid oxidase 1 (HAO1)	[202]
Givosira	2019	rare blood disorder, treat acute hepatic porphyria,	Liver	aminolevulinate synthase 1 (ALAS1)	[203]
Patisiran	2018	treat adult patients' polyneuropathy caused by familial transthyretin-mediated amyloidosis	Liver	transthyretin (TTR)	[204]

The nanomedicines also improve the ADME profile as well as targeting multiple sites and tumor microenvironments. Translating co-delivery of chemo-drugs with siRNA has been investigated in vitro and in vivo. Cancer therapeutics have been the story of increasing the efficacy of treatment and reducing the complications post-treatment. Researchers continually pitched for the co-delivery of chemotherapeutics drugs, which proved clinically effective.[198] The major merits of co-delivery include synergistic effect, reduced toxicity, and overcoming multidrug resistance (MDR).

Table 3 *Polymeric nanosystems for delivery of siRNA/ peptide and Drugs*

Sr No.	Nano system	Peptide	RNAi gene targets	Drug	Target	Reference
1	PEGylated cationic nanoliposomes	YSA peptide (ephrin type-A receptor 2)	JNK-interacting protein	doxorubicin	osteosarcoma multidrug resistance	[205]
2	peptide-siRNA nanoparticles	p5RHH (NH2-VLTTGLPALISWI RRRHRRHC-COOH)	c-Jun N-terminal kinases 2		atherothrombosis (Invitro-invivo study)	[206]
3	nano-graphene oxide	poly-l-lysine	vascular endothelial growth factor	doxorubicin (DOX)	Cancer therapy (invitro/ invivo)	[207]
4	polyethyleneimine-block-polylactic acid pH-responsive		survivin	paclitaxel	lung cancer therapy	[208]
8	collagen-modified siRNAs gold nanoparticle		siRNAs epidermal growth factor		Lung cancer	[209]
9	mucin1 aptamer-conjugated chitosan nanoparticles		cMET siRNA	docetaxel	breast cancer (in Vitro)	[210]

10	aptamer-conjugated grapefruit-derived nanovectors		P-glycoprotein	doxorubicin	Multidrug resistance colon cancer (in vitro)	[211]
11	Dexamethasone (Dex)-loaded PHEA-g-C18-Arg8 (PCA) nanoparticles	arginine 8 (Arg8) peptide	connexin 26 [Cx26] siRNA	Dexamethasone	inner ear therapy	[212]

The nanomedicines also improve the ADME profile as well as targeting multiple sites and tumor microenvironments. Translating co-delivery of chemo-drugs with siRNA has been investigated in vitro and in vivo. The degradation nature of siRNA leads to protection with the help of nanocarriers to effectively transfect intra-cellular. Chemo-drugs such as curcumin, paclitaxel, and doxorubicin have already proven their effectiveness clinically. When these chemo-drugs are combined with siRNA therapeutics enhances the chemo resistance of cancer cells.[213] The dose requirements for co-delivery are lower than mono-therapy for the same desired therapeutic effect. This could ultimately benefit all types and stages of cancers. Many co-delivery systems are in the clinical pipelines and most of them completed the Phase I trial and may be approved by the FDA.[214]. Polymer- siRNA delivery systems must shield siRNA from degradation in contact of biological fluids and should facilitate cellular uptake to the targeted cells, and trigger endosomal escape[215]

1.2.4.10. Challenges in siRNA designing and anticancer therapy.

From a design point of view, the marketed siRNA therapeutics are chemically modified along with the support of delivery platforms such as GalNAc to enhance efficacy and therapeutic effect on hepatocytes.[216] Given the numerous delivery platforms such as gold nanoparticles, polymer, RGD peptides, lipids, exosomes could be used to target various tumors and

cancers.[217] these platforms will overcome the three major barriers: entry, endosomal escape, and efficacy.

1.3. RESEARCH GAP AND MOTIVATION

1.3.4. **Requirement in development of nerve growth and angiogenesis promoting polymers.** The tissues, organs, and cells employed in regenerative medicine are intricate and challenging to produce on a large scale. Although, stem cells hold promise in regenerative medicine, but self-renewal, multipotency, and tumorigenicity during and post-therapy are also raising concerns. Thus, the difficulty in developing and translating successful stem cell-based medicines. Researchers have used the recombinant growth factor to deliver for regenerative applications, however, it holds the some consequences. In the same manner that VEGF controls several signaling pathways, high-level VEGF is also linked to cancer. The main obstacles to practical applications are expensive preparations, a need for a particular target tissue retention, and undesirable side effects. .[144] Therefore, development of stimulatory material based on amino-acid based polymer is an important challenge.

1.3.5. Requirement in development of anti-angiogenic and anticancer polymers.

Optimizing treatment delivery and selection of delivery systems is challenging. Acquired resistance and tumor heterogeneity are likely the primary factors impeding precision oncology's progress. The selection of the most effective delivery mechanism to specifically target cancer cells is one of the major issues facing siRNA therapy. Genome integration is a constant concern associated with gene therapy. The high possibilities of being neutralized by the immune system for deciding on correct doses to be administered to patient and controlled release to reach only particular targets.[218].

As per the recent clinical trial data of RNA therapeutics majority of works are focused towards the expression analysis and very few are explored towards the. The siRNA-based therapeutics has now reached patients only in the past 5 years with the approval of the FDA. Though pharmacotherapy was developed for rare or orphan diseases, the goal to reach broader diseases such as hemophilia, and optic neuropathy are in late phase -3 clinical trials.[219] This lower number of approvals for siRNA therapeutics may be due to various challenges mentioned earlier. Moreover, none of the siRNA anticancer therapeutics have gone to phase-3 trials. One of the major issues in clinical trials was non-specificity towards tumor tissues with deposition in other organs. Another issue arises due to the lack of enough pharmacodynamics data from human clinical trials.[170] based on the above motivations and gape of the earlier work, the following are the primary objective of this dissertation as follows.

1.4. MAJOR OBJECTIVES

Based on the above research gap the major objective was development of "*Amino acid-based polymeric hydrogel and nanoparticles for neuroregeneration and siRNA-drug loaded formulation for anticancer applications*" divided into four objectives as listed below:

Objective I. Synthesis of Poly (N-acryloyl glycine-b-acrylamide) hydrogel and Evaluation for its neuroprotective and neuroregenerative properties.

Objective II. Modulation of p(NAG-b-Ac) bio-Polymer by glutamate to synthesize for poly(N-acryloyl glycine)-co-(acrylamide)-(N-acryloyl glutamate) for angiogenesis and neuroregeneration.

Objective III. Comparative assessment of glycine, n-acryloyl glycine and glycine based Anti-cancer poly(N-acryloyl glycine-b-acrylamide) hydrogel for its geometric properties and

induce cell killing in poor prognosis heparanase driven malignancies *through in silico and in vitro approach*.

Objective IV: Synthesis and characterization of bio-polymeric p(NALE –co- NAPA) nanoparticles for siRNA and drug delivery for the treatment of cancer.

REFERENCES:

1. AbdulKarim, H., et al., *Novel poly(ester amide) derived from tyrosine amino acid for targeted pulmonary drug delivery of fluticasone propionate.* Journal of Applied Polymer Science, 2023. **140**(13): p. e53672.
2. Hobert, J.A., et al., *Quantitative analysis of urine acylglycines by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS): Reference intervals and disease specific patterns in individuals with organic acidemias and fatty acid oxidation disorders.* Clinica Chimica Acta, 2021. **523**: p. 285-289.
3. Khan, W., et al., *Biodegradable Polymers Derived From Amino Acids.* Macromolecular Bioscience, 2011. **11**(12): p. 1625-1636.
4. Ahmed, W., M. Karabaliev, and C. Gao, *Taking chiral polymers toward immune regulation.* Journal of Polymer Science, 2022. **60**(15): p. 2213-2224.
5. Green, D.W., et al., *Chiral Biomaterials: From Molecular Design to Regenerative Medicine.* Advanced Materials Interfaces, 2016. **3**(6): p. 1500411.
6. Hancu, G. and A. Modroiu *Chiral Switch: Between Therapeutical Benefit and Marketing Strategy*. Pharmaceuticals, 2022. **15**, DOI: 10.3390/ph15020240.
7. Higashi, N., R. Sonoda, and T. Koga, *Thermo-responsive amino acid-based vinyl polymers showing widely tunable LCST/UCST behavior in water*. RSC Advances, 2015. **5**(83): p. 67652-67657.
8. Yadav, R., et al., *Biophysical study on the phase transition behaviour of biocompatible thermoresponsive polymer influenced by tryptophan-based amino acid ionic liquids.* Polymer, 2021. **228**: p. 123871.
9. Bauri, K., M. Nandi, and P. De, *Amino acid-derived stimuli-responsive polymers and their applications.* Polymer Chemistry, 2018. **9**(11): p. 1257-1287.

10. Kim, G., et al., *The role of oxidative stress in neurodegenerative diseases, Exp.* Neurobiology, 2015. **24**: p. 325.
11. Zewen Liu, 2 Tingyang Zhou,1,3 Alexander C. Ziegler,4 Peter Dimitrion,4 and Li Zuo1,3, *Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications.* Oxidative Medicine and Cellular Longevity, 2017: p. 11.
12. Zhang, X., X. Peng, and S.W. Zhang, *1 - Biodegradable medical polymers: Fundamental sciences*, in *Science and Principles of Biodegradable and Bioresorbable Medical Polymers*, X. Zhang, Editor. 2017, Woodhead Publishing. p. 1-33.
13. Gohil, S.V., et al., *Chapter 8 - Polymers and Composites for Orthopedic Applications*, in *Materials for Bone Disorders*, S. Bose and A. Bandyopadhyay, Editors. 2017, Academic Press. p. 349-403.
14. Gupta, S.S., et al., *Amino acid derived biopolymers: Recent advances and biomedical applications.* International Journal of Biological Macromolecules, 2021. **188**: p. 542-567.
15. Guo, Y., et al., *Solvent-Free Preparation of Thermally Stable Poly(urethane-imide) Elastomers.* ACS Applied Polymer Materials, 2023. **5**(6): p. 4517-4524.
16. Tardy, A., et al., *Radical Ring-Opening Polymerization: Scope, Limitations, and Application to (Bio)Degradable Materials.* Chemical Reviews, 2017. **117**(3): p. 1319-1406.
17. Perrier, S., *50th Anniversary Perspective: RAFT Polymerization—A User Guide.* Macromolecules, 2017. **50**(19): p. 7433-7447.
18. Danaei, M., et al. *Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems*. Pharmaceutics, 2018. **10**, DOI: 10.3390/pharmaceutics10020057.
19. Lorandi, F., M. Fantin, and K. Matyjaszewski, *Atom Transfer Radical Polymerization: A Mechanistic Perspective.* Journal of the American Chemical Society, 2022. **144**(34): p. 15413-15430.
20. Lamontagne, H.R. and B.H. Lessard, *Nitroxide-Mediated Polymerization: A Versatile Tool for the Engineering of Next Generation Materials.* ACS Applied Polymer Materials, 2020. **2**(12): p. 5327-5344.

21. Chen, Y., et al., *Recent Advances in Living Cationic Polymerization with Emerging Initiation/Controlling Systems.* Macromolecular Rapid Communications, 2021. **42**(13): p. 2100148.
22. Hirao, A., R. Goseki, and T. Ishizone, *Advances in Living Anionic Polymerization: From Functional Monomers, Polymerization Systems, to Macromolecular Architectures.* Macromolecules, 2014. **47**(6): p. 1883-1905.
23. Sutthasupa, S., M. Shiotsuki, and F. Sanda, *Recent advances in ring-opening metathesis polymerization, and application to synthesis of functional materials.* Polymer Journal, 2010. **42**(12): p. 905-915.
24. Nayak, K., et al., *Side-chain amino-acid-based polymers: self-assembly and bioapplications.* Polymer International, 2022. **71**(4): p. 411-425.
25. Elumalai, K., S. Srinivasan, and A. Shanmugam, *Review of the efficacy of nanoparticle-based drug delivery systems for cancer treatment.* Biomedical Technology, 2024. **5**: p. 109-122.
26. Tian, H., et al., *Enhancing the therapeutic efficacy of nanoparticles for cancer treatment using versatile targeted strategies.* Journal of Hematology & Oncology, 2022. **15**(1): p. 132.
27. Vinogradov, S.V., et al., *Polyplex Nanogel formulations for drug delivery of cytotoxic nucleoside analogs.* J Control Release, 2005. **107**(1): p. 143-57.
28. Guterres, S.S., M.P. Alves, and A.R. Pohlmann, *Polymeric nanoparticles, nanospheres and nanocapsules, for cutaneous applications.* Drug Target Insights, 2007. **2**: p. 147-57.
29. Mitchell, M.J., et al., *Engineering precision nanoparticles for drug delivery.* Nature Reviews Drug Discovery, 2021. **20**(2): p. 101-124.
30. Madaan, K., et al., *Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues.* J Pharm Bioallied Sci, 2014. **6**(3): p. 139-50.
31. Wang, J., et al., *Dendrimer-based drug delivery systems: history, challenges, and latest developments.* Journal of Biological Engineering, 2022. **16**(1): p. 18.
32. Cao, S., et al., *Amphiphilic AIEgen-polymer aggregates: Design, self-assembly and biomedical applications.* Aggregate, 2022. **3**(1): p. e128.

33. Wang, J., et al., *Self-Assembly Properties and Aggregation Behavior of Amphiphilic Water-Dispersed Polyester with Different Contents of Sulfonate Groups in an Aqueous Solution.* Langmuir, 2023. **39**(20): p. 7006-7016.
34. Banik, B.L., P. Fattahi, and J.L. Brown, *Polymeric nanoparticles: the future of nanomedicine.* WIREs Nanomedicine and Nanobiotechnology, 2016. **8**(2): p. 271-299.
35. Gardner, C.M., et al., *Poly(methyl vinyl ether-alt-maleic acid) Polymers for Cell Encapsulation.* Journal of Biomaterials Science, Polymer Edition, 2011. **22**(16): p. 2127-2145.
36. Venkataraman, S., et al., *The effects of polymeric nanostructure shape on drug delivery.* Advanced Drug Delivery Reviews, 2011. **63**(14): p. 1228-1246.
37. Kim, W., et al., *Plasma-assisted multiscale topographic scaffolds for soft and hard tissue regeneration.* npj Regenerative Medicine, 2021. **6**(1): p. 52.
38. Kim, H.N., et al., *Nanotopography-guided tissue engineering and regenerative medicine.* Adv Drug Deliv Rev, 2013. **65**(4): p. 536-58.
39. Chen, W., et al., *Nanotopography regulates motor neuron differentiation of human pluripotent stem cells.* Nanoscale, 2018. **10**(7): p. 3556-3565.
40. Tong, R. and J. Cheng, *Anticancer Polymeric Nanomedicines.* Polymer Reviews, 2007. **47**(3): p. 345-381.
41. Schmaljohann, D., *Thermo- and pH-responsive polymers in drug delivery.* Advanced Drug Delivery Reviews, 2006. **58**(15): p. 1655-1670.
42. Zhang, Y., et al., *Temperature- and pH-Responsive Star Polymers as Nanocarriers with Potential for in Vivo Agrochemical Delivery.* ACS Nano, 2020. **14**(9): p. 10954-10965.
43. Roy, S.G. and P. De, *pH responsive polymers with amino acids in the side chains and their potential applications.* Journal of Applied Polymer Science, 2014. **131**(20).
44. Yoshida, T., et al., *pH- and ion-sensitive polymers for drug delivery.* Expert Opin Drug Deliv, 2013. **10**(11): p. 1497-513.
45. Bazban-Shotorbani, S., et al., *Revisiting structure-property relationship of pH-responsive polymers for drug delivery applications.* Journal of Controlled Release, 2017. **253**: p. 46-63.

46. Kocak, G., C. Tuncer, and V. Bütün, *pH-Responsive polymers.* Polymer Chemistry, 2017. **8**(1): p. 144-176.
47. Singh, J. and P. Nayak, *pH-responsive polymers for drug delivery: Trends and opportunities.* Journal of Polymer Science, 2023. **61**(22): p. 2828-2850.
48. Yang, Y., et al., *Antimicrobial cationic polymers: from structural design to functional control.* Polymer Journal, 2018. **50**(1): p. 33-44.
49. Samal, S.K., et al., *Cationic polymers and their therapeutic potential.* Chemical Society Reviews, 2012. **41**(21): p. 7147-7194.
50. Numata, K., *Poly(amino acid)s/polypeptides as potential functional and structural materials.* Polymer Journal, 2015. **47**(8): p. 537-545.
51. Longo, G.S., N.A. Pérez-Chávez, and I. Szleifer, *How protonation modulates the interaction between proteins and pH-responsive hydrogel films.* Current Opinion in Colloid & Interface Science, 2019. **41**: p. 27-39.
52. Khare, A.R. and N.A. Peppas, *Swelling/deswelling of anionic copolymer gels.* Biomaterials, 1995. **16**(7): p. 559-567.
53. Tang, J., et al., *Swelling Behaviors of Hydrogels with Alternating Neutral/Highly Charged Sequences.* Macromolecules, 2020. **53**(19): p. 8244-8254.
54. Jiang, Y., et al., *Quantitating Endosomal Escape of a Library of Polymers for mRNA Delivery.* Nano Letters, 2020. **20**(2): p. 1117-1123.
55. Evans, B.C., et al., *An anionic, endosome-escaping polymer to potentiate intracellular delivery of cationic peptides, biomacromolecules, and nanoparticles.* Nature Communications, 2019. **10**(1): p. 5012.
56. Pei, D. and M. Buyanova, *Overcoming Endosomal Entrapment in Drug Delivery.* Bioconjug Chem, 2019. **30**(2): p. 273-283.
57. Degors, I.M.S., et al., *Carriers Break Barriers in Drug Delivery: Endocytosis and Endosomal Escape of Gene Delivery Vectors.* Accounts of Chemical Research, 2019. **52**(7): p. 1750-1760.
58. Winkeljann, B., D.C. Keul, and O.M. Merkel, *Engineering poly- and micelleplexes for nucleic acid delivery – A reflection on their endosomal escape.* Journal of Controlled Release, 2023. **353**: p. 518-534.

59. Wang, S., *pH-Responsive amphiphilic carboxylate polymers: Design and potential for endosomal escape.* Frontiers in Chemistry, 2021. **9**: p. 645297.
60. Kaneko, M., et al., *Anticancer Activity of Cell-Penetrating Redox Phospholipid Polymers.* ACS Macro Letters, 2021. **10**(7): p. 926-932.
61. Liu, B., et al., *Hydrogen peroxide-responsive anticancer hyperbranched polymer micelles for enhanced cell apoptosis.* Polymer Chemistry, 2015. **6**(18): p. 3460-3471.
62. Park, S.-B., et al., *Poly (glutamic acid): Production, composites, and medical applications of the next-generation biopolymer.* Progress in Polymer Science, 2021. **113**: p. 101341.
63. Wei, M., et al., *Preparation of pH-Responsive Poly(gamma-glutamic acid) Hydrogels by Enzymatic Cross-Linking.* ACS Biomater Sci Eng, 2022. **8**(2): p. 551-559.
64. Kim, M.H., et al., *Enzymatically Cross-Linked Poly(gamma-glutamic acid) Hydrogel with Enhanced Tissue Adhesive Property.* ACS Biomater Sci Eng, 2020. **6**(5): p. 3103-3113.
65. Zhang, W., et al., *A tough and self-healing poly (L-glutamic acid)-based composite hydrogel for tissue engineering.* Journal of Materials Chemistry B, 2018. **6**(42): p. 6865-6876.
66. Petrov, P.D., et al., *Biodegradable polymer network encapsulated polyplex for DNA delivery.* RSC Advances, 2013. **3**(11): p. 3508-3511.
67. Zhang, J., et al., *Efficacy and mechanism of poloxamine-assisted polyplex transfection.* J Gene Med, 2013. **15**(8-9): p. 271-81.
68. Dinari, A., et al., *Synthesis and Characterization of a Nano-Polyplex system of GNRs-PDMAEA-pDNA: An Inert Self-Catalyzed Degradable Carrier for Facile Gene Delivery.* Scientific Reports, 2018. **8**(1): p. 8112.
69. Pandey, M., et al., *Targeted and Enhanced Antimicrobial Inhibition of Mesoporous ZnO–Ag2O/Ag, ZnO–CuO, and ZnO–SnO2 Composite Nanoparticles.* ACS Omega, 2021. **6**(47): p. 31615-31631.
70. Borjihan, Q. and A. Dong, *Design of nanoengineered antibacterial polymers for biomedical applications.* Biomaterials Science, 2020. **8**(24): p. 6867-6882.

71. Barman, S., et al., *Amino Acid Conjugated Polymers: Antibacterial Agents Effective against Drug-Resistant Acinetobacter baumannii with No Detectable Resistance.* ACS Applied Materials & Interfaces, 2019. **11**(37): p. 33559-33572.
72. Xie, Y.-Y., et al., *Structure-Dependent Antibacterial Activity of Amino Acid-Based Supramolecular Hydrogels.* Colloids and Surfaces B: Biointerfaces, 2020. **193**: p. 111099.
73. Zhu, J., et al., *Self-assembly of amino acid-based random copolymers for antibacterial application and infection treatment as nanocarriers.* Journal of Colloid and Interface Science, 2019. **540**: p. 634-646.
74. Serrano-Aroca, Á., et al., *Scaffolds in the microbial resistant era: Fabrication, materials, properties and tissue engineering applications.* Materials Today Bio, 2022. **16**: p. 100412.
75. Labusca, L., D.D. Herea, and K. Mashayekhi, *Stem cells as delivery vehicles for regenerative medicine-challenges and perspectives.* World J Stem Cells, 2018. **10**(5): p. 43-56.
76. Dubey, A., H. Vahabi, and V. Kumaravel, *Antimicrobial and Biodegradable 3D Printed Scaffolds for Orthopedic Infections.* ACS Biomaterials Science & Engineering, 2023. **9**(7): p. 4020-4044.
77. Chavda, V.P., et al. *Antibody–Biopolymer Conjugates in Oncology: A Review*. Molecules, 2023. **28**, DOI: 10.3390/molecules28062605.
78. Srivastava, A., et al., *Polymer-antibody fragment conjugates for biomedical applications.* Progress in Polymer Science, 2014. **39**(2): p. 308-329.
79. Najjar, A., A. Najjar, and R. Karaman *Newly Developed Prodrugs and Prodrugs in Development; an Insight of the Recent Years*. Molecules, 2020. **25**, DOI: 10.3390/molecules25040884.
80. Figueiredo, P.R., R.D. González, and A.T.P. Carvalho, *Insights into the Degradation of Polymer–Drug Conjugates by an Overexpressed Enzyme in Cancer Cells.* Journal of Medicinal Chemistry, 2023. **66**(4): p. 2761-2772.
81. Li, Y., et al., *Anticancer Polymers via the Biginelli Reaction.* ACS Macro Letters, 2020. **9**(9): p. 1249-1254.

82. Sliwoski, G., et al., *Computational methods in drug discovery.* Pharmacol Rev, 2014. **66**(1): p. 334-95.
83. Sadybekov, A.V. and V. Katritch, *Computational approaches streamlining drug discovery.* Nature, 2023. **616**(7958): p. 673-685.
84. Zhou, P., C. Yuan, and X. Yan, *Computational approaches for understanding and predicting the self-assembled peptide hydrogels.* Current Opinion in Colloid & Interface Science, 2022. **62**: p. 101645.
85. Ramezanpour, M., et al., *Computational and experimental approaches for investigating nanoparticle-based drug delivery systems.* Biochimica et Biophysica Acta (BBA) - Biomembranes, 2016. **1858**(7, Part B): p. 1688-1709.
86. Wang, W., et al., *Computational pharmaceutics - A new paradigm of drug delivery.* Journal of Controlled Release, 2021. **338**: p. 119-136.
87. Li, A., H.S. Muddana, and M.K. Gilson, *Quantum Mechanical Calculation of Noncovalent Interactions: A Large-Scale Evaluation of PMx, DFT, and SAPT Approaches.* Journal of Chemical Theory and Computation, 2014. **10**(4): p. 1563-1575.
88. De Vivo, M., et al., *Role of Molecular Dynamics and Related Methods in Drug Discovery.* Journal of Medicinal Chemistry, 2016. **59**(9): p. 4035-4061.
89. Wong-ekkabut, J. and M. Karttunen, *The good, the bad and the user in soft matter simulations.* Biochimica et Biophysica Acta (BBA) - Biomembranes, 2016. **1858**(10): p. 2529-2538.
90. Badar, M.S., et al., *Molecular Dynamics Simulations: Concept, Methods, and Applications*, in *Transdisciplinarity*, N. Rezaei, Editor. 2022, Springer International Publishing: Cham. p. 131-151.
91. van Mourik, T., M. Bühl, and M.P. Gaigeot, *Density functional theory across chemistry, physics and biology.* Philos Trans A Math Phys Eng Sci, 2014. **372**(2011): p. 20120488.
92. Ye, N., Z. Yang, and Y. Liu, *Applications of density functional theory in COVID-19 drug modeling.* Drug Discov Today, 2022. **27**(5): p. 1411-1419.
93. Ye, N., Z. Yang, and Y. Liu, *Applications of density functional theory in COVID-19 drug modeling.* Drug Discovery Today, 2022. **27**(5): p. 1411-1419.

94. Adekoya, O.C., et al. *Application of DFT Calculations in Designing Polymer-Based Drug Delivery Systems: An Overview*. Pharmaceutics, 2022. **14**, DOI: 10.3390/pharmaceutics14091972.
95. Torres, P.H.M., et al., *Key Topics in Molecular Docking for Drug Design.* Int J Mol Sci, 2019. **20**(18).
96. Abdolmaleki, A., F. Shiri, and J.B. Ghasemi, *Chapter 11 - Use of Molecular Docking as a Decision-Making Tool in Drug Discovery*, in *Molecular Docking for Computer-Aided Drug Design*, M.S. Coumar, Editor. 2021, Academic Press. p. 229-243.
97. Meng, X.Y., et al., *Molecular docking: a powerful approach for structure-based drug discovery.* Curr Comput Aided Drug Des, 2011. **7**(2): p. 146-57.
98. Schmidt, C.E. and J.B. Leach, *Neural Tissue Engineering: Strategies for Repair and Regeneration.* Annual Review of Biomedical Engineering, 2003. **5**(1): p. 293-347.
99. Varadarajan, S.G., et al., *Central nervous system regeneration.* Cell, 2022. **185**(1): p. 77-94.
100. Tian, L., M.P. Prabhakaran, and S. Ramakrishna, *Strategies for regeneration of components of nervous system: scaffolds, cells and biomolecules.* Regenerative Biomaterials, 2015. **2**(1): p. 31-45.
101. Struzyna, L.A., et al., *Restoring nervous system structure and function using tissue engineered living scaffolds.* Neural Regeneration Research, 2015. **10**(5).
102. Min, Q., D.B. Parkinson, and X.P. Dun, *Migrating Schwann cells direct axon regeneration within the peripheral nerve bridge.* Glia, 2021. **69**(2): p. 235-254.
103. Gwak, S.J., et al., *RhoA knockdown by cationic amphiphilic copolymer/siRhoA polyplexes enhances axonal regeneration in rat spinal cord injury model.* Biomaterials, 2017. **121**: p. 155-166.
104. Mao, A.S. and D.J. Mooney, *Regenerative medicine: Current therapies and future directions.* Proc Natl Acad Sci U S A, 2015. **112**(47): p. 14452-9.
105. *Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015.* Lancet Neurol, 2017. **16**(11): p. 877-897.

106. Gourie-Devi, M., *Epidemiology of neurological disorders in India: review of background, prevalence and incidence of epilepsy, stroke, Parkinson's disease and tremors.* Neurol India, 2014. **62**(6): p. 588-98.
107. Fan, Y., et al., *Burden and trends of brain and central nervous system cancer from 1990 to 2019 at the global, regional, and country levels.* Archives of Public Health, 2022. **80**(1): p. 209.
108. Mattson, M.P. and T. Magnus, *Ageing and neuronal vulnerability.* Nat Rev Neurosci, 2006. **7**(4): p. 278-94.
109. Arnsten, A.F.T., *Stress signalling pathways that impair prefrontal cortex structure and function.* Nature Reviews Neuroscience, 2009. **10**(6): p. 410-422.
110. Preston, Alison R. and H. Eichenbaum, *Interplay of Hippocampus and Prefrontal Cortex in Memory.* Current Biology, 2013. **23**(17): p. R764-R773.
111. Smith, M.A., *Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors.* Behav Brain Res, 1996. **78**(1): p. 25-36.
112. de Haan, W., et al., *Activity dependent degeneration explains hub vulnerability in Alzheimer's disease.* PLoS Comput Biol, 2012. **8**(8): p. e1002582.
113. Oakley, R.H. and J.A. Cidlowski, *The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease.* J Allergy Clin Immunol, 2013. **132**(5): p. 1033-44.
114. Dong, X.X., Y. Wang, and Z.H. Qin, *Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases.* Acta Pharmacol Sin, 2009. **30**(4): p. 379-87.
115. Marra, F., et al. *An Overview of Mitochondrial Protein Defects in Neuromuscular Diseases*. Biomolecules, 2021. **11**, DOI: 10.3390/biom11111633.
116. Oury, J., et al., *Mechanism of disease and therapeutic rescue of Dok7 congenital myasthenia.* Nature, 2021. **595**(7867): p. 404-408.
117. Fralish, Z., et al., *Neuromuscular Development and Disease: Learning From in vitro and in vivo Models.* Frontiers in Cell and Developmental Biology, 2021. **9**.
118. Paß, T., R.J. Wiesner, and D. Pla-Martín, *Selective Neuron Vulnerability in Common and Rare Diseases-Mitochondria in the Focus.* Front Mol Biosci, 2021. **8**: p. 676187.

119. Mancuso, M.R., F. Kuhnert, and C.J. Kuo, *Developmental angiogenesis of the central nervous system.* Lymphat Res Biol, 2008. **6**(3-4): p. 173-80.
120. Xiong, Y., A. Mahmood, and M. Chopp, *Angiogenesis, neurogenesis and brain recovery of function following injury.* Curr Opin Investig Drugs, 2010. **11**(3): p. 298-308.
121. Madelaine, R., et al., *MicroRNA-9 Couples Brain Neurogenesis and Angiogenesis.* Cell Reports, 2017. **20**(7): p. 1533-1542.
122. Teng, H., et al., *Coupling of angiogenesis and neurogenesis in cultured endothelial cells and neural progenitor cells after stroke.* J Cereb Blood Flow Metab, 2008. **28**(4): p. 764-71.
123. Vassilopoulos, A., et al., *Treatment Approaches for Functional Neurological Disorders in Children.* Curr Treat Options Neurol, 2022. **24**(2): p. 77-97.
124. Idoia, G., et al., *Therapeutic Opportunities and Delivery Strategies for Brain Revascularization in Stroke, Neurodegeneration, and Aging.* Pharmacological Reviews, 2022. **74**(2): p. 439.
125. Font, M.A., A. Arboix, and J. Krupinski, *Angiogenesis, neurogenesis and neuroplasticity in ischemic stroke.* Curr Cardiol Rev, 2010. **6**(3): p. 238-44.
126. Kim, T.A., L. Chen, and S. Ge, *The interplay of neurovasculature and adult hippocampal neurogenesis.* Neurosci Lett, 2021. **760**: p. 136071.
127. Aloe, L., et al., *Nerve Growth Factor: A Focus on Neuroscience and Therapy.* Curr Neuropharmacol, 2015. **13**(3): p. 294-303.
128. Huang, E.J. and L.F. Reichardt, *Neurotrophins: roles in neuronal development and function.* Annu Rev Neurosci, 2001. **24**: p. 677-736.
129. Johnson, K.E. and T.A. Wilgus, *Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair.* Adv Wound Care (New Rochelle), 2014. **3**(10): p. 647-661.
130. Emanueli, C., et al., *Nerve Growth Factor Promotes Angiogenesis and Arteriogenesis in Ischemic Hindlimbs.* Circulation, 2002. **106**(17): p. 2257-2262.
131. Idrisova, K.F., et al., *Application of neurotrophic and proangiogenic factors as therapy after peripheral nervous system injury.* Neural Regen Res, 2022. **17**(6): p. 1240-1247.

132. Rosa, R.G., et al., *Growth Factor Stimulation Improves the Structure and Properties of Scaffold-Free Engineered Auricular Cartilage Constructs.* PLOS ONE, 2014. **9**(8): p. e105170.
133. Wang, Z., et al., *Novel biomaterial strategies for controlled growth factor delivery for biomedical applications.* NPG Asia Materials, 2017. **9**(10): p. e435-e435.
134. Okesola, B.O., et al., *Growth-Factor Free Multicomponent Nanocomposite Hydrogels That Stimulate Bone Formation.* Advanced Functional Materials, 2020. **30**(14): p. 1906205.
135. Ren, X., et al., *Growth Factor Engineering Strategies for Regenerative Medicine Applications.* Frontiers in Bioengineering and Biotechnology, 2020. **7**.
136. Duman, R.S., *Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression.* Dialogues Clin Neurosci, 2009. **11**(3): p. 239-55.
137. Lukic-Panin, V., et al., *Prevention of neuronal damage by calcium channel blockers with antioxidative effects after transient focal ischemia in rats.* Brain Research, 2007. **1176**: p. 143-150.
138. Boni, R., et al., *Current and novel polymeric biomaterials for neural tissue engineering.* Journal of Biomedical Science, 2018. **25**(1): p. 90.
139. Mallapragada, S.K. and J.B. Recknor, *POLYMERIC BIOMATERIALS FOR NERVE REGENERATION*, in *Advances in Chemical Engineering*. 2004, Academic Press. p. 47-74.
140. Akhtar, A., et al., *Emerging polymeric biomaterials and manufacturing-based tissue engineering approaches for neuro regeneration-A critical review on recent effective approaches.* Smart Materials in Medicine, 2023. **4**: p. 337-355.
141. Laranjeira, M., et al., *3D Mimicry of Native-Tissue-Fiber Architecture Guides Tendon-Derived Cells and Adipose Stem Cells into Artificial Tendon Constructs.* Small, 2017. **13**(31): p. 1700689.
142. Li, B., et al., *Revealing the Immunogenic Risk of Polymers.* Angewandte Chemie International Edition, 2018. **57**(42): p. 13873-13876.
143. Yuan, Z., et al., *Hidden hydrophobicity impacts polymer immunogenicity*. Chemical Science, 2023. **14**(8): p. 2033-2039.

144. Lu, J., et al., *Enhanced angiogenesis by the hyaluronic acid hydrogels immobilized with a VEGF mimetic peptide in a traumatic brain injury model in rats.* Regenerative Biomaterials, 2019. **6**(6): p. 325-334.

145. Kim, D.H., et al., *Enhancing neurogenesis and angiogenesis with target delivery of stromal cell derived factor-1α using a dual ionic pH-sensitive copolymer.* Biomaterials, 2015. **61**: p. 115-125.

146. Hao, Z., et al., *Tetramethylpyrazine promotes angiogenesis and nerve regeneration and nerve defect repair in rats with spinal cord injury.* Heliyon, 2023. **9**(11): p. e21549.

147. González-Pérez, F., et al., *Protease-Sensitive, VEGF-Mimetic Peptide, and IKVAV Laminin-Derived Peptide Sequences within Elastin-Like Recombinamer Scaffolds Provide Spatiotemporally Synchronized Guidance of Angiogenesis and Neurogenesis.* Advanced Healthcare Materials, 2022. **11**(22): p. 2201646.

148. Álvarez, Z., et al., *Neurogenesis and vascularization of the damaged brain using a lactate-releasing biomimetic scaffold.* Biomaterials, 2014. **35**(17): p. 4769-4781.

149. Meng, X.-Y., et al., *Vascular endothelial growth factor-loaded poly-lactic-co-glycolic acid nanoparticles with controlled release protect the dopaminergic neurons in Parkinson's rats.* Chemical Biology & Drug Design, 2020. **95**(6): p. 631-639.

150. Kalashnikova, I., et al., *Optimization and characterization of miRNA-129-5p-encapsulated poly (lactic-co-glycolic acid) nanoparticles to reprogram activated microglia.* Nanoscale Advances, 2023. **5**(13): p. 3439-3452.

151. Si, H.B., et al., *Control-released basic fibroblast growth factor-loaded poly-lactic-co-glycolic acid microspheres promote sciatic nerve regeneration in rats.* Exp Ther Med, 2017. **13**(2): p. 429-436.

152. Jeong, J.H., et al., *Protective Effect of Cholic Acid-Coated Poly Lactic-Co-Glycolic Acid (PLGA) Nanoparticles Loaded with Erythropoietin on Experimental Stroke.* Journal of Nanoscience and Nanotechnology, 2019. **19**(10): p. 6524-6533.

153. Wang, L., et al., *A biomimetic piezoelectric scaffold with sustained Mg2+ release promotes neurogenic and angiogenic differentiation for enhanced bone regeneration.* Bioactive Materials, 2023. **25**: p. 399-414.

154. Juriga, D., et al. *Analysis of Three-Dimensional Cell Migration in Dopamine-Modified Poly(aspartic acid)-Based Hydrogels*. Gels, 2022. **8**, DOI: 10.3390/gels8020065.

155. Song, H.K., et al., *Micropatterns of positive guidance cues anchored to polypyrrole doped with polyglutamic acid: A new platform for characterizing neurite extension in complex environments.* Biomaterials, 2006. **27**(3): p. 473-484.
156. Fayzullin, A., et al., *Phenotypic and Expressional Heterogeneity in the Invasive Glioma Cells.* Transl Oncol, 2019. **12**(1): p. 122-133.
157. Sung, H., et al., *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.* CA: A Cancer Journal for Clinicians, 2021. **71**(3): p. 209-249.
158. Almansour, N.M., *Triple-Negative Breast Cancer: A Brief Review About Epidemiology, Risk Factors, Signaling Pathways, Treatment and Role of Artificial Intelligence.* Frontiers in Molecular Biosciences, 2022. **9**.
159. Tamimi, A.F. and M. Juweid, *Epidemiology and Outcome of Glioblastoma*, in *Glioblastoma*, S. De Vleeschouwer, Editor. 2017, Codon Publications

Copyright: The Authors.: Brisbane (AU).

160. Damaghi, M. and R. Gillies, *Phenotypic changes of acid-adapted cancer cells push them toward aggressiveness in their evolution in the tumor microenvironment.* Cell Cycle, 2017. **16**(19): p. 1739-1743.
161. Janardan, S.K. and D.S. Wechsler, *Caught in the In-Between: Challenges in Treating Adolescents and Young Adults With Cancer.* JCO Oncology Practice, 2021. **17**(6): p. 299-301.
162. Mahmoud, R., P. Ordóñez-Morán, and C. Allegrucci *Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness*. Cancers, 2022. **14**, DOI: 10.3390/cancers14174280.
163. Wei, Z., et al., *Metabolism of Amino Acids in Cancer.* Frontiers in Cell and Developmental Biology, 2021. **8**.
164. Bhutia, Y.D., et al., *Amino Acid Transporters in Cancer and Their Relevance to "Glutamine Addiction": Novel Targets for the Design of a New Class of Anticancer Drugs.* Cancer Research, 2015. **75**(9): p. 1782-1788.
165. Saito, Y. and T. Soga, *Amino acid transporters as emerging therapeutic targets in cancer.* Cancer Science, 2021. **112**(8): p. 2958-2965.

166. Tavares, M.R., et al., *Polymer-Based Drug-Free Therapeutics for Anticancer, Anti-Inflammatory, and Antibacterial Treatment.* Macromolecular Bioscience, 2021. **21**(8): p. 2100135.
167. Zhong, G., et al., *Polymers with distinctive anticancer mechanism that kills MDR cancer cells and inhibits tumor metastasis.* Biomaterials, 2019. **199**: p. 76-87.
168. Takahashi, H., et al., *Anticancer polymers designed for killing dormant prostate cancer cells.* Scientific Reports, 2019. **9**(1): p. 1096.
169. Charbe, N.B., et al., *Small interfering RNA for cancer treatment: overcoming hurdles in delivery.* Acta pharmaceutica Sinica. B, 2020. **10**(11): p. 2075-2109.
170. Hattab, D., A.M. Gazzali, and A. Bakhtiar, *Clinical Advances of siRNA-Based Nanotherapeutics for Cancer Treatment.* Pharmaceutics, 2021. **13**(7).
171. Tian, Z., et al., *Insight Into the Prospects for RNAi Therapy of Cancer*. Front Pharmacol, 2021. **12**: p. 644718.
172. Chernikov, I.V., et al., 2023.
173. Bernstein, E., et al., *Role for a bidentate ribonuclease in the initiation step of RNA interference.* Nature, 2001. **409**(6818): p. 363-366.
174. Grishok, A., et al., *Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing.* Cell, 2001. **106**(1): p. 23-34.
175. Hammond, S.M., et al., *Argonaute2, a link between genetic and biochemical analyses of RNAi.* Science, 2001. **293**(5532): p. 1146-1150.
176. Schwarz, D.S., et al., *Asymmetry in the assembly of the RNAi enzyme complex.* Cell, 2003. **115**(2): p. 199-208.
177. Adele Alagia, a.A.F.J., ‡*c Anna Avi˜o,n′, et al., *Exploring PAZ/30-overhang interaction to improve siRNA specificity. A combined experimental and modeling study†.* Chem. Sci, 2018. **9**: p. 2074.
178. Tomari, Y., et al., *A protein sensor for siRNA asymmetry.* Science, 2004. **306**(5700): p. 1377-80.
179. Ohrt, T., et al., *Intracellular localization and routing of miRNA and RNAi pathway components.* Current topics in medicinal chemistry, 2012. **12**(2): p. 79-88.

180. Ipsaro, J.J. and L. Joshua-Tor, *From guide to target: molecular insights into eukaryotic RNA-interference machinery.* Nat Struct Mol Biol, 2015. **22**(1): p. 20-8.
181. Boland, A., et al., *Crystal structure and ligand binding of the MID domain of a eukaryotic Argonaute protein.* EMBO reports, 2010. **11**(7): p. 522-527.
182. Xu, L., et al., *Structure-based design of novel chemical modification of the 3'-overhang for optimization of short interfering RNA performance.* Biochemistry, 2015. **54**(5): p. 1268-77.
183. Hu, B., et al. *Therapeutic siRNA: state of the art*. Signal transduction and targeted therapy, 2020. **5**, 101 DOI: 10.1038/s41392-020-0207-x.
184. Lin, X., et al., *siRNA-mediated off-target gene silencing triggered by a 7 nt complementation.* Nucleic Acids Res, 2005. **33**(14): p. 4527-35.
185. Hu, B., et al., *Therapeutic siRNA: state of the art.* Signal Transduction and Targeted Therapy, 2020. **5**(1): p. 101.
186. Cho, W.G., et al., *Small interfering RNA-induced TLR3 activation inhibits blood and lymphatic vessel growth.* Proc Natl Acad Sci U S A, 2009. **106**(17): p. 7137-42.
187. Kaushal, A., *Innate immune regulations and various siRNA modalities.* Drug Deliv Transl Res, 2023. **13**(11): p. 2704-2718.
188. Broering, R., et al., *Chemical modifications on siRNAs avoid Toll-like-receptor-mediated activation of the hepatic immune system in vivo and in vitro.* Int Immunol, 2014. **26**(1): p. 35-46.
189. Soutschek, J., et al., *Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs.* Nature, 2004. **432**(7014): p. 173-8.
190. Dar, S.A., et al., *siRNAmod: A database of experimentally validated chemically modified siRNAs.* Scientific Reports, 2016. **6**(1): p. 20031.
191. Selvam, C., et al., *Therapeutic potential of chemically modified siRNA: Recent trends.* Chem Biol Drug Des, 2017. **90**(5): p. 665-678.
192. Deleavey, G.F., J.K. Watts, and M.J. Damha, *Chemical modification of siRNA.* Curr Protoc Nucleic Acid Chem, 2009. **Chapter 16**: p. Unit 16.3.
193. Oh, Y.-K. and T.G. Park, *siRNA delivery systems for cancer treatment.* Advanced Drug Delivery Reviews, 2009. **61**(10): p. 850-862.

194. Ahn, I., C.S. Kang, and J. Han, *Where should siRNAs go: applicable organs for siRNA drugs.* Experimental & Molecular Medicine, 2023. **55**(7): p. 1283-1292.
195. Kurakula, H., et al., *Emergence of Small Interfering RNA-Based Gene Drugs for Various Diseases.* ACS Omega, 2023. **8**(23): p. 20234-20250.
196. Isazadeh, H., et al., *Advances in siRNA delivery approaches in cancer therapy: challenges and opportunities.* Molecular Biology Reports, 2023. **50**(11): p. 9529-9543.
197. Jain, D., et al., *Nano-formulated siRNA-based therapeutic approaches for cancer therapy.* Nano Trends, 2023. **1**: p. 100006.
198. Gao, Q., et al., *Opportunities and challenges for co-delivery nanomedicines based on combination of phytochemicals with chemotherapeutic drugs in cancer treatment.* Advanced Drug Delivery Reviews, 2022. **188**: p. 114445.
199. Syed, Y.Y., *Nedosiran: First Approval.* Drugs, 2023. **83**(18): p. 1729-1733.
200. Keam, S.J., *Vutrisiran: First Approval.* Drugs, 2022. **82**(13): p. 1419-1425.
201. Lamb, Y.N., *Inclisiran: First Approval.* Drugs, 2021. **81**(3): p. 389-395.
202. Scott, L.J. and S.J. Keam, *Lumasiran: First Approval.* Drugs, 2021. **81**(2): p. 277-282.
203. Scott, L.J., *Givosiran: First Approval.* Drugs, 2020. **80**(3): p. 335-339.
204. Kalola, U.K. and M.V. Pellegrini, *Patisiran*, in *StatPearls [Internet]*. 2023, StatPearls Publishing.
205. Haghiralsadat, F., et al., *Preparation of PEGylated cationic nanoliposome-siRNA complexes for cancer therapy.* Artif Cells Nanomed Biotechnol, 2018. **46**(sup1): p. 684-692.
206. Pan, H., et al., *Anti-JNK2 peptide–siRNA nanostructures improve plaque endothelium and reduce thrombotic risk in atherosclerotic mice.* International Journal of Nanomedicine, 2018: p. 5187-5205.
207. Sun, Q., et al., *Doxorubicin and anti-VEGF siRNA co-delivery via nano-graphene oxide for enhanced cancer therapy in vitro and in vivo.* Int J Nanomedicine, 2018. **13**: p. 3713-3728.
208. Jin, M., et al., *Smart polymeric nanoparticles with pH-responsive and PEG-detachable properties for co-delivering paclitaxel and survivin siRNA to enhance antitumor outcomes.* Int J Nanomedicine, 2018. **13**: p. 2405-2426.

209. Yu, A.Y.-H., et al., *Epidermal growth factor receptors siRNA-conjugated collagen modified gold nanoparticles for targeted imaging and therapy of lung cancer*. Materials Today Advances, 2021. **12**: p. 100191.
210. Jafari, R., et al., *Anti-Mucin1 Aptamer-Conjugated Chitosan Nanoparticles for Targeted Co-Delivery of Docetaxel and IGF-1R siRNA to SKBR3 Metastatic Breast Cancer Cells.* Iran Biomed J, 2019. **23**(1): p. 21-33.
211. Tang, Z., et al., *Aptamer-conjugated and doxorubicin-loaded grapefruit-derived nanovectors for targeted therapy against HER2(+) breast cancer*. J Drug Target, 2020. **28**(2): p. 186-194.
212. Yoon, J.Y., et al., *The effect of dexamethasone/cell-penetrating peptide nanoparticles on gene delivery for inner ear therapy.* Int J Nanomedicine, 2016. **11**: p. 6123-6134.
213. Ashrafizadeh, M., et al., *Progress in Natural Compounds/siRNA Co-delivery Employing Nanovehicles for Cancer Therapy.* ACS Comb Sci, 2020. **22**(12): p. 669-700.
214. Yadav, D.N., et al., *Recent Advancements in the Design of Nanodelivery Systems of siRNA for Cancer Therapy*. Mol Pharm, 2022. **19**(12): p. 4506-4526.
215. Singh, P., et al., *Chapter 6 - siRNA polymer conjugates for the delivery of RNAi therapeutics for the treatment of Parkinson's disease*, in *Nanomedical Drug Delivery for Neurodegenerative Diseases*, A.K. Yadav, R. Shukla, and S.J.S. Flora, Editors. 2022, Academic Press. p. 81-98.
216. Zuckerman, J.E. and M.E. Davis, *Clinical experiences with systemically administered siRNA-based therapeutics in cancer.* Nature Reviews Drug Discovery, 2015. **14**(12): p. 843-856.
217. Friedrich, M. and A. Aigner, *Therapeutic siRNA: State-of-the-Art and Future Perspectives.* BioDrugs, 2022. **36**(5): p. 549-571.
218. Pucci, C., C. Martinelli, and G. Ciofani, *Innovative approaches for cancer treatment: current perspectives and new challenges.* Ecancermedicalscience, 2019. **13**: p. 961.
219. Zhang, M.M., et al., *The growth of siRNA-based therapeutics: Updated clinical studies.* Biochemical Pharmacology, 2021. **189**: p. 114432.