# ***Abstract***

The study's goal of this dissertation was the synthesis of amino acid-based polymeric hydrogel to investigate neurogenic potential and nanoparticles for anticancer application. Poly (amino acid) polymers are not studied well for their neuroregenrative potential and only a few polymers are known as anticancer polymer, which shows anticancer properties. Amino acid-derived polymers contain various –NH, -CO-NH and -COOH functional groups, which offer the key advantages (i) introduction of new imaging, molecular targeting and drug moieties, (ii) Modulation of swelling and rheological behaviour for the 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 a readily metabolized product from the body. These properties facilitate polymeric hydrogel and nanoparticles to qualify for biomedical applications such as neuroregeneration, siRNA and drug delivery. Overall, the neuroregeneration and anticancer activities with the polymeric hydrogel and nanoparticles have been studied.

In brief, **Chapter-1** covers the introduction to biopolymers, hydrogel, nanoparticle/nanocapsules, neuroregeneration, nucleic acids, RNAs (siRNAs), anticancer drugs etc. and challenges along with motivation for study design. Similarly, **Chapter-2** provided the more information on study design, synthesis and characterization process used to establish the novel co-polymeric hydrogel and nanoparticles for neuroregenration and anticancer application. The fundamental principles of the experiment procedures employed for the characterisation of produced polymeric nanocapsules/hydrogel have been thoroughly described along with siRNA designing, encapsulation and stability protocols.

Insilco/ invtro investigation for the use of neuroregeneration, and cancer therapy have been thoroughly described for these particles.

Neuron repair is one of the challenging task in the regenerative research are due to inadequate knowledge of CNS development, pathophysiology, and the lack of multifunctional properties of biomaterials which limits the development of clinically effective neuron regenerative biomaterials along with limited therapeutic success rate. Vascular damage, increased inflammations, oxidative stress and insufficient blood supply are the leading causes of death and neurological disability. Increased burden of stress, and continuously increased burden of cancer are co-relative phenomena, as cancer patients diagnosed with peripheral neuropathy as a side effect. And continuous increased stress and genetic alteration increasing the risk of glioblastoma. Hence to fulfill the goal of the investigation and based on obtained results **Chapter-3** is further divided into four parts.

In **Part:1** Evaluation of Poly(N-acryloyl glycine-acrylamide) (p(NAG-b-Ac)) hydrogel for neuroprotective and neuroregenerative applications. It is found that high swelling behavior, viscoelastic nature, 2.9 kPa to 3.7 kPa moduli of p(NAG-b-Ac) provide the 3D extra cellular mimetic microenvironment to the neuronal cells. The presence of various functional groups such as –COOH and amide group in p(NAG-b-A) hydrogel scavenges the free radicals via oxidation reaction and protects neuronal damage from oxidative stress. Insilico results shows p(NAG-co-Ac) interaction with GSK3β leads to GSK3β inhibition and nerve regeneration. This property made it, a clinically relevant material for neuronal regenerative applications, since it provides the physical cue and significantly boosts the neurite outgrowth along with maintaining the microtubule integrity in neuronal cells.

**Furthermore, In Part 2 of Chapter 3** is focused on the Modulation of p(NAG-b-Ac) bio-Polymer by glutamate to synthesize for Poly(N-acryloyl glycine)-co-(acrylamide)-(N-

acryloyl glutamate) for building the angiogenic and neurogenic material. The p(NAG-Ac-NAE) hydrogel induces higher pro-angiogenic activity compared to the p(NAG-co-Ac) hydrogel and shows neurogenic potential in primary neuronal cells. Neo-angiogenic activity of glutamate is reduced by the development of p(NAG-Ac-NAE) hydrogel and showed cytotoxic behaviour on aggressive cancer cell lines. The results are clear shreds of evidence that the p(NAG-Ac-NAE) hydrogel is angiogenic, while p(NAG-co-Ac) hydrogel is anti-angiogenic in nature. The results further indicate that the angiogenic potential could arise due to the presence of excitatory neurotransmitter glutamate imprinting. Further, the p(NAG-Ac-NAE) and p(NAG-b-A) hydrogels show a protective role via scavenging ROS generation and mitochondrial membrane depolarization in oxidative stress conditions. We also observed a higher cellular proliferation in p(NAG-Ac-NAE) hydrogel compared to the p(NAG-b-A) hydrogel, due to the excitatory role of glutamate and cooperative phenomena of glycine and glutamate. A significant increase in cell proliferation is observed in p(NAG-Ac-NAE) hydrogel, whereas a decrease in proliferation is observed in the case of p(NAG-b-A) hydrogel-treated PC12 cells. Our results further demonstrated that the p(NAG-Ac-NAE) hydrogel promotes stable cytoskeleton network formation. Thus, the obtained results revealed that the synthesized p(NAG-Ac-NAE) hydrogel shows excellent biocompatibility, hemocompatibility, favorable mechanical stability, and neuroprotective, which favours the angiogenic and neurogenic characteristics together with neuro-regenerative potential.

**In Part 3 of Chapter 3**, the main objective is to address poor prognosis of heparanase-driven malignancies by developing the anticancer polymer for drug delivery. For this, *in silico and DFT* approaches were used to predict the biological activity and heparanase inhibitory activity of bio-polymeric poly(N-acryloyl glycine-acrylamide) hydrogel for anticancer application.. The geometrical and electronic properties of Glycine, N-

acryloylglycine monomer (NAG), and polymeric units of poly [(N-acryloylglycine)-co-(acrylamide)] p(NAG-co-Ac) are rationalized by density functional theory (DFT) with B3LYP/ 6-311g* and experimental evidences have been provided for their uses in cancer therapy. The Frontier Molecular Orbital theory (FMO) reveals that the introduction of acrylamide/divinylbenzene in the polymer has increased biological activities by lowering the band gap energy. Furthermore, the linear and cross-linked co-polymeric rearrangements shows the protease inhibitory activities and heparanase inhibitory activity by interacting with the heparanase binding domain II (HBDII) with more affinity dock score of -11.08 kcal $mol^{-1}$ in nanomolar Ki. Experimentally, it is found that the p(NAG-co-Ac) hydrogel shows anti-proliferative and migratory inhibitory activity towards invasive aggressive cancer such as triple-negative breast cancer (TNBC) by intracellular ROS production and leads the programmed cell death. Additionally, the p(NAG-co-Ac) hydrogel exhibited anti-angiogenic behaviour in the *in-ovo* system. The p(NAG-co-Ac) hydrogel showed higher cytotoxicity towards aggressive cancer cell lines than the normal healthy cell lines. Lack of cell-killing effect of p(NAG-co-Ac) hydrogel in the MCF-7 cell line corroborated the results of heparanase inhibition as heparanase expression in MCF-7 is lower than the MDAMB-231 and LN229. Further, p(NAG-co-Ac) hydrogel inhibits the migration of MDA-MB-231 and LN299 cells and induces apoptotic death in both cell lines. In LN299 cells, the glycine induces the necrotic death which may be due to the excessive ROS generation, while p(NAG-co-Ac) hydrogel induces the programmed cell death by modulating the ROS production at a therapeutic level. Angiogenesis and heparanase activity is a co-relative behavior and the highest expression of heparanase in esophageal cancers, bladder cervical and colorectal cancers etc. made a p(NAG-co-Ac) to the potential biomaterial for anti-cancer application.

Furthermore, in **Part 4 of Chapter 3**, the objective was focused: to Synthesize and characterizing the polymeric nanoparticles for siRNA and drug delivery for cancer treatment Multi-targeting phenylalanine and Lucien-based, morphologically spherical and amorphous acrylate (p(NAPA-co-LME)) copolymeric nanoparticle were synthesized and characterized for physical and biological properties. The side chain containing amino acid-derived polymer exhibits significant properties such as stimuli responsiveness, self-assembling characteristic behavior, and the presence of amino group and -COO favor the interactions with drug and small molecule siRNA. The formation of phenylalanine and Lucien polymer and its copolymer along with the biocompatibility in L929 and PC12 cells induce the cellular internalization through the LAT1 transporter. The presence of aromatic rings in the polymer enhance the loading efficiency of siPOL2RA up to 70% and high loading efficiency observed for drug along with the silencing of POL2RA. Chemical modification and entrapment in nanoparticles induce the serum stability of particles. Furthermore, In Chapter 4, finally, we can conclude that p(NAG-co-Ac) and p(NAG-co-NAE) is the future potential material for neuroregeneration. Similarly, the p p(NAG-co-Ac) hydrogel and p(NAPA-co-LME) are the synergistic drug delivery carrier for heparanase-driven malignancies**.** In Conclusion, this siRNA/drug loaded p(NAPA-co-LME) polymeric nanoparticle separately or in combination could be a multi-targeting tool in advanced stage cancer therapy in a targeted manner.