Abstract

Amino acid-based copolymer nanoparticles (NPs) offer promising impact on organ-specific drug delivery and tissue regeneration. These amphiphilic copolymers form stable nanostructures under physiological conditions and can efficiently encapsulate therapeutic drugs irrespective of their phobicity. Their biodegradability and structural mimicry of natural proteins support in cancer therapy and tissue regeneration, leveraging both passive (EPR effect) and active targeting strategies. On account of the above facts, this dissertation work aimed to develop a library of NPs based on two amino acids (Glycine and Phenylalanine) and subsequently they are modified for their therapeutic applications.

A library molecules of organ targeting drug delivery systems (OTDDS) with *N*-acryloyl glycine (NAG) and *N*-acryloyl (*L*-phenylalanine methyl ester) (NAPA) [p(NAG-*co*-NAPA)$_{(x:y)}$] have been designed, and screened for their *in vitro* biocompatibility and *in vivo* organ targeting efficiency. Each composition of OTDDS can target multiple organs such as liver, heart, lungs, breast and kidney up to a different extent. From this library, as the p(NAG-*co*-NAPA)$_{(1:4)}$ NPs with a size of 160-210nm target breast with a high extent relative to other organs, it has been optimized for the TNBC treatment. In addition to this, from *in silico* study 14 TNBC-related genes were identified, leading to the use of a dual-drug formulation: Dihydroartemisinin (DHA, targeting 6 genes) and piperine (targeting 8 genes). This optimized system achieved high therapeutic efficacy against TNBC (IC_{50} 350 µg/mL), validating the copolymer NPs as a robust platform for targeted combination therapy. Further, to enhance the TNBC targeting efficiency, p(NAG-*co*-NAPA)$_{(1:4)}$ NPs are conjugated with folic acid which showed improved, pH-dependent drug release in acidic tumor microenvironments and still a low IC_{50} value (i.e.,280µg/mL) in *in vitro*. This TNBC inhibition at such a low concentration by treatment with folate conjugated combined nanoformulation is occurred due to the mitochondrial membrane potential mediated cell death by arresting the G2-M phase of the cell cycle. Moreover, gelatine zymography and RT-PCR results demonstrated anti-angiogenic, anti-invasion and anti-proliferative properties of Co-NFs via downregulating the TNBC specific genes including DNMT3B, EGFR, Ki67, STAT3, Bcl2 and CDK2, along with upregulating caspase 9 expressions. Together, these results highlight the nanoformulation's strong anti-TNBC potential and effectiveness in targeting tumor biology at multiple levels.

Further, the crosslinkers used in the synthesis of above mentioned library NPs are highly active and tend to bind with the endothelial cells, body protein, metal ions, enzymes and lipids which can hinder multiple systemic cellular activities. In this line, a self-assembled amphiphilic block copolymer NPs i.e., p(NAG-*co*-NAPA)$_{wc}$ has been synthesized by obviating chemical crosslinker. The structural evolution of self-assembled NAG-NAPA at different temperatures have been confirmed through the molecular dynamic (MD) simulation, which corroborated the structural stability of the copolymer NPs at physiological temperature (37^{o}C). The effect of p(NAG-*co*-NAPA)$_{wc}$ NPs on cell migration assay are complementary to CEMA assay results and revealed its dose and time dependent angiogenic properties. This p(NAG-*co*-NAPA)$_{wc}$ NPs is hemocompatible, non-irritant and help in cell proliferation and tissue regeneration. The *in vivo* wound healing study demonstrated ~97% of wound site has been filled within 13 days of post treatment, whereas for control it is only ~80%. Furthermore, this *in vivo* wound healing efficiency of p(NAG-*co*-NAPA)$_{wc}$ NPs has been validated through RT-PCR results, i.e., downregulating TNF-α and IL-1β; and upregulating PECAM-1 and VEGF-A. These modulations in key inflammatory markers is an evidence for its therapeutic potential in wound healing and tissue regeneration.

Further to assess the angiogenesis, a new method has been developed by using Gray-level co-occurrence matrix (GLCM)-based texture features image processing tool, which could be co-related with more reliable parameters. To establish this method, form OTDDS library p(NAG-*co*-NAPA)$_{(1:1)}$ NPs with an average particle size of 100-130 nm in dia. with porous structure has been used as a model polymer NPs. This p(NAG-*co*-NAPA) NPs is biocompatible and exhibited cell proliferation up to ~115-120% with L929 (mouse fibroblast), HUVEC (Human umbilical endothelial cells) and Raw 264.7 macrophages. In the 2nd step, the pro-angiogenic properties of p(NAG-*co*-NAPA)$_{(1:1)}$ NPs are investigated through tube formation assay and '*in ovo*' model followed by analysis of images by Angiotool to predict its regenerative efficacy. Then ~1000 microscopic images of newly formed blood vessels are segmented using adaptive thresholding and used to compute seven texture features including number of pixels in blood vessels, entropy, mean, energy, contrast, dissimilarity, and variance. These results are correlated with biological angiogenesis parameters by establishing a definitive, objective standard for regenerative efficacy prediction.

Altogether, this dissertation manifests that amino acid-based copolymer NPs can be finely tuned for organ-selective drug delivery, multi-modal cancer therapy, and for tissue regeneration with broad impacts across precision therapeutics and nanobioengineering.