List of 10 best papers of the applicant highlighting the important discoveries/contributions

1. Raval N, Jogi H, Gondaliya P, Kalia K, **Tekade RK*,** **Method and its Composition for encapsulation, stabilization, and delivery of siRNA in Anionic polymeric nanoplex: An In vitro- In vivo Assessment.** ***NATURE: Scientific Reports*****,** 2019, 9, 16047, DOI: 10.1038/s41598-019-52390-4 **(IF: 4.6).**

Highlight of this research	**Contribution and Role**
Despite their high potency, specificity, and therapeutic potential, the full-fledged utility of siRNA is predominantly limited to *in vitro* setup. To date, Onpattro is the only USFDA approved siRNA therapeutics available in the clinic. The lack of a reliable *in vivo* siRNA delivery carrier remains a foremost obstacle towards the clinical translation of siRNA therapeutics. To address the obstacles associated with siRNA delivery, we tested a dendrimer-templated polymeric approach involving a USFDA approved biopolymer (albumin) for *in vitro* as well as *in vivo* delivery of siRNA. **Patent obtained from this research:** **Title of the patent:** Method of loading and stabilization of siRNA in negatively charged Polymer for cytosolic delivery of same. **Indian patent, Patent Application No: # 201921019898 (Date: 20/05/2019).** **Inventor Name: Tekade RK,** Raval N, Jogi H, Gondaliya P, Kalia K **The approach developed herein could be extrapolated to other gene therapeutics and other kidney-related diseases.**	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development • Project planning and execution as mentor

2. Raval N, Gondaliya P, Tambe V, Kalia K, **Tekade RK*.** **Engineered nanoplex mediated targeted miRNA delivery to rescue dying podocytes in diabetic nephropathy.** ***International Journal of Pharmaceutics.*** 2021, 605, 120842. DOI: 10.1016/j.ijpharm.2021.120842 **(IF: 5.875)**

Highlight of this research	**Contribution and Role**
In the diabetic nephropathic condition, miRNA-30a is directly and primarily suppressed by hyperglycemic kidney induced Notch signaling pathway leads to podocyte damage and apoptosis. Thus, transferring the exogenous miRNA-30a to podocytes might improve albuminuria as well as podocytes injury. The deprived stability, poor targetability, and low specificity in vivo are critical limitations to attain this objective**.** This investigation reports the specific and efficient delivery of miRNA-30a mimic via cyclo(RGDfC)-gated polymeric-nanoplexes with dendrimer templates to alleviate podocyte conditions. The nanoplexes able to protect RNase enzyme	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development • Project planning and execution as mentor

and to exhibit greater cellular uptake viaαvβ3 receptor selective binding in HG treated podocytes. The nanoplexes up-regulated the expression level of miRNA-30a and repress the elevated Notch-1 signaling in HG exposed podocytes. The critical results of in vivo experimentation attribute marked suppression of Notch-1 in streptozotocin (STZ) induced diabetic C57BL/6 mice and reduced glomerular expansion and fibrosis in the glomerular area. Developed nanoplexes represents an efficient platform for the targeted delivery of exogenous miRNA to podocytes.	

3. Raval N, Jogi H, Gondaliya P, Kalia K, **Tekade RK*, Cyclo-RGD truncated Polymeric Nanoconstruct with Dendrimeric templates for targeted HDAC4 Gene Silencing in a Diabetic Nephropathy Mouse Model". *Molecular Pharmaceutics*,** 2021, 18, 2, 641–666 DOI: 10.1021/acs.molpharmaceut.0c00094 **(IF: 4.5).**

Highlight of this research	**Contribution and Role**
The HDAC-inhibitors have emerged as a critical class of therapeutic agents in DN; however, the currently available HDAC4-inhibitors are mostly nonselective in nature as well as inhibit multiple HDACs. RNA interference of HDAC4 (HDAC4 siRNA) has shown immense promise, but the clinical translation has been impeded due to lack of a targeted, specific, and in vivo applicable delivery modality. In the present investigation, we examined Cyclo(RGDfC) (cRGD) truncated polymeric nanoplex with dendrimeric templates for targeted HDAC4 Gene Silencing. The developed nanoplex exhibited enhanced encapsulation of siRNA and offered superior protection against serum RNase nucleases degradation. The nanoplex was tested on podocytes (in vitro), wherein it showed selective binding to the αvβ3 integrin receptor, active cellular uptake, and significant in vitro gene silencing. The in vivo experiments showed remarkable suppression of the HDAC4 and inhibition in the progression of renal fibrosis in the Streptozotocin (STZ) induced DN C57BL/6 mice model. Histopathological and toxicological studies revealed nonsignificant abnormality/toxicity with the nanoplex. Conclusively, nanoplex was found as a promising tactic for targeted therapy of podocytes and could be extended for other kidney-related ailments.	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development • Project planning and execution as mentor

List of 10 best papers of the applicant highlighting the important discoveries/contributions

4. Pradhan D, Tambe V, Raval N, Gondalia P, Bhattacharya P, Kalia K, **Tekade RK***. **Dendrimer Grafted Albumin Nanoparticles for the Treatment of Post Cerebral Stroke Damages: A Proof of Concept Study.** ***Colloids and Surfaces B: Biointerfaces,*** 2019, 184, 110488 DOI: 10.1016/j.colsurfb.2019.110488 **(IF: 5.3).**

Highlight of this research	**Contribution and Role**
Citicoline (CIT) is reported to enhance the acetylcholine secretion in the brain and also helps in membrane repair and regeneration. However, the poor BBB permeation of CIT results in lower levels of CIT in the brain. This demands the development of a suitable delivery platform to completely realize the therapeutic benefit of CIT in stroke therapy. This investigation reports the synthesis and characterization of second generation (2.0 G) dendrimer Amplified Albumin (dAA) biopolymer by FTIR, MALDI-TOF, and surface charge (mV). Further, the synthesized biopolymer has been utilized to develop a CIT nanoformulation using a commercially translatable one-pot process. Release of CIT from biopolymer was performed within an acetate buffer at pH 5 and Phosphate buffer at pH 7.4. Further, we investigated the ability of biopolymer to permeate BBB by in vitro permeability assay in bEnd.3 cells. MTT assay of CIT-dAA-NP, CIT-ANP, and 2.0 G PAMAM dendrimers was performed in bEnd.3 cells. Therapeutic efficacy of the synthesized biopolymer was determined by VEGF gene expression within an in vitro hypoxia model in PC12 cells. Thus, this investigation resulted in biopolymers that can be used to deliver any therapeutic agent by altering the permeability of the BBB. Also, cationization by dendrimer grafting is one such strategy that may be used to cationize any other negatively charged polymer, such as albumin. The synthesized biopolymer is not limited to deliver molecules to the brain, but can also be used to increase the loading of negatively-charged drug molecules, siRNA, or any other oligonucleotide.	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development • Project planning and execution as mentor

5. Tambe V, Raval N, Gondaliya P, Bhattacharya P, Kalia K, **Tekade RK*. To investigate fit-to-purpose nanocarrier for non-invasive drug delivery to posterior segment of eye.** ***J. Drug Delivery Science and Technology.*** 2021, 61, 102222. DOI: 10.1016/j.jddst.2020.102222 **(IF: 3.981)**

Highlight of this research	**Contribution and Role**
Diseases of the posterior segment of the eye are difficult to treat due to lesser bioavailability of therapeutics at the posterior segment of the eye. Current clinical interventions involve administering drugs via invasive routes (Intravitreal, Retrobulbar, or Peribulbar) that bear the least patient compliance. Several nanocarriers strategies have shown a	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development

remarkable potential to deliver the loaded therapeutic to the posterior tissues of the eye. However, no one platform report was available to demarcate the most effective delivery system out of the recommended approaches. This investigation aimed at exploring a suitable and efficient non-invasive topically administrable nanocarriers system for the delivery of the drug to the posterior segment of the eye. Dexamethasone (DEX, a corticosteroid used for ocular inflammation) was selected as a model drug. The nanocarriers were formulated (size ~120 nm) and studied their potential at a common platform to deliver the drug to the posterior segment of the eye. The nanocarriers were analyzed for their in vitro drug release profile in simulated tear fluid (STF) depicting sustained release of DEX up to 24 hrs, ex-vivo corneal permeability, using excised goat cornea, cytotoxicity potential using human retinal pigment epithelium ARPE-19 cell lines, HET CAM assay to evaluate ocular irritancy, electrical resistance measurement across monolayers of Rabbit corneal epithelial cells SIRC and ARPE-19 cells and stability profiles, real-time qPCR IL-6 gene expression in ARPE-19 inflammation model. Ex-vivo corneal permeability demonstrated that the highest percentage of DEX was permeated by DEX-NLCs and lowest by DEX-SLNs. All the nanocarriers except DEX-CUBs depicted no cytotoxicity in ARPE-19 cells. All the nanocarriers depicted the change in electrical resistance measurement across monolayers of Rabbit corneal epithelial cells SIRC and ARPE-19 cells. They were also found to reduce the levels of IL-6 in gene expression assay depicting successful invitro delivery to ARPE-19 cells. However, only NLCs and NMFs were selected further as they demonstrated better potential to permeate after ex vivo and in vitro permeability studies. Further, in-vivo fluorescence imaging studies in Wistar rats were also performed with coumarin-6 loaded nanocarriers to deduce the effective and suitable candidate to deliver a drug to the posterior segment of the eye with the highest biosafety and permeability.	• Project planning and execution as mentor

6. Moeendarbari S, **Tekade RK,** Mulgaonkar A, Christensen P, et al. **Theranostic Nanoseeds for Efficacious Internal Radiation Therapy of Unresectable Solid Tumors.** ***Nature Scientific Reports,*** 2016, 6, 20614. DOI:10.1038/srep20614 **(IF- 5.578).**

Highlight of this research	**Contribution and Role**
Malignant tumors are considered "unresectable" if they are adhering to vital structures or the surgery would cause irreversible damages to the patients. Though a variety of cytotoxic drugs and radiation therapies are currently available in clinical practice to treat such tumor masses, these therapeutic modalities are always associated with substantial side effects.	• **Role:** Postdoctoral research work • Carried out at University of Hawaii, USA

Here, we report an injectable nanoparticle-based internal radiation source that potentially offers more efficacious treatment of unresectable solid tumors without significant adverse side effects. Using a highly efficient incorporation procedure, palladium-103, a brachytherapy radioisotope in clinical practice, was coated to monodispersed hollow gold nanoparticles with a diameter about 120 nm, to form (103)Pd@Au nanoseeds. The therapeutic efficacy of (103)Pd@Au nanoseeds were assessed when intratumorally injected into a prostate cancer xenograft model. Five weeks after a single-dose treatment, a significant tumor burden reduction (>80%) was observed without noticeable side effects on the liver, spleen and other organs. Impressively, **>95%** nanoseeds were retained inside the tumors as monitored by Single Photon Emission Computed Tomography (SPECT) with the gamma emissions of (103)Pd. These findings show that this nanoseed-based brachytherapy has the potential to provide a theranostic solution to unresectable solid tumors.	• Idea, hypothesis, Project planning and execution

7. Pandey PK, Maheshwari R, Raval N, Gondaliya P, Kalia K, **Tekade RK*. Nanogold-core multifunctional dendrimer for pulsatile chemo-, photothermal- and photodynamic-therapy of rheumatoid arthritis.** ***J. Colloid Interface Science*** 2019, 22, 544:61-77. DOI: 10.1016/j.jcis.2019.02.073 **(IF: 8.2).**

Highlight of this research	**Contribution and Role**
This investigation reports a novel nanoGold-core multifunctional dendrimer for pulsatile chemo-, photothermal- and photodynamic- therapy of rheumatoid arthritis (RA). Architecturally, the nanocomposites comprised of a nanoGold (Au) at the focal whose surface is functionalized by hydroxy-terminated thiolated-dendrons following Au-thiol bond formation to produce nanoGold-core multifunctional dendrimer (Au-DEN). The surface hydroxyl groups of Au-DEN were then conjugated with methotrexate (MTX; a disease-modifying first line anti-rheumatic drug; DMARD; 74.29 ± 0.48% loading) to form Au-DEN-MTX-NPs (Particle size: 100.15 ± 28.36 nm; poly dispersibility index, PDI: 0.39 ± 0.02; surface zeta potential, ζ: -22.45 ± 1.06 mV). MTX was strategically selected to serve as an anti-rheumatic DMARD as well as a targeting ligand to attain selective localization of the formulation in arthritic tissue via folate receptors upregulated on arthritic tissues. The docking study was performed to confirm the viable binding efficiency of MTX towards β-folate receptors that are overexpressed on arthritic tissues taking folic acid as a reference standard. The IR780, a NIR active bioactive was also loaded in Au-DEN-MTX NPs to offer photothermal benefit upon irradiation with NIR laser (wavelength: 808 nm). The hypothesis was tested by elucidation of in vitro	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development • Project planning and execution as mentor

drug release profile, photothermal activity, cellular uptake (Fluorescence and confocal laser scanning microscopy; CLSM), cell viability assay (MTT protocol) and Intracellular reactive oxygen species (ROS) generation in mouse macrophage RAW264.7 cells and Lipopolysaccharide (LPS) activated RAW264.7 cells. Furthermore, the hemolytic toxicity and stability studies were also investigated to determine the blood compatibility as well as ideal storage condition of NPs. The outcome of this investigations presents developed multifunctional targeted NPs to be potential therapeutics for the improved treatment of RA. The approach can also be applied to other clinical interventions involving countering inflammatory conditions.	

8. Gadeval A, Maheshwari R, Raval N, Kalyane D, Kalia K, **Tekade RK*. Green graphene nanoplates for combined photo-chemo-thermal therapy of triple-negative breast cancer.** ***Nanomedicine (Lond).*** 2020, 15, 581-601 DOI: 10.2217/nnm-2019-0380 **(IF: 5.3)**.

Highlight of this research	**Contribution and Role**
Green graphene oxide (GO) nanoplates, which are reduced and stabilized by quercetin and guided by folate receptors (quercetin reduced and loaded GO nanoparticles-folic acid [FA]), were developed to mediate combined photo-chemo-thermal therapy of triple-negative breast cancer. Materials & methods: Modified Hummers method was used for the synthesis of GO followed by its reduction using quercetin, FA was then conjugated as a targeting ligand. A cytotoxicity assay, apoptosis assay and cellular uptake assay were performed in vitro in MDA-MB-231 cell line with and without irradiation of a near-infrared 808 nm laser. Quercetin reduced and loaded GO nanoparticles-FA showed significantly high cellular uptake ($p < 0.001$) and cytotoxic effects in MDA-MB-231 cells, which was even more prominent under the situation of near-infrared 808 nm laser irradiation, making it a potential option for treating triple-negative breast cancer.	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development • Project planning and execution as mentor

9. Patharkar A, Raval N, Kalyane D, Tambe V, Anup N, More N, Kapusetti G, Kalia K, **Tekade RK*. Glucosamine-conjugated nanoseeds for chemo-magneto hyperthermia therapy of cancer.** ***J. Drug Delivery Science and Technology.*** 2021, 61, 102295. DOI: 10.1016/j.jddst.2020.102295 **(IF: 3.981)**

Highlight of this research	**Contribution and Role**
Nowadays, magnetic hyperthermia is being extensively used in the treatment of cancer therapy due to its ability to kill cancer cells through the thermal effect.	• **Role:** Principal Investigator (PI) & Corresponding author • Idea and hypothesis development

This study reports the synthesis of multifunctional Doxorubicin (DOX) loaded Gadolinium/Cobalt@Iron oxide-Dendrimer-Glucosamine-Nanoseed (Gd/Co@IO-D-G-NS) for the treatment of prostate cancer. Furthermore, glucosamine ligand was attached to enhance the effectiveness of nanoseeds via targeting glucose transporters. The release profile of DOX from Gd/Co@IO-D-G-NS nanoseeds was observed to be pH-dependent. The developed nanosseds showed acceptable hemocompatibility, and were able to produce combined hyperthermia and chemotherapeutic effect in vitro in PC3 cells. Moreover, cellular uptake studies revealed that nanoseeds were effectively uptaken by cancer cells through receptor-mediated endocytosis. It is expected that the outcome of this study will assist in progressing the know-how towards the development of nanoseeds for chemo-photothermal therapy of resectable cancers, including prostate and breast, to naming a few.	• Project planning and execution as mentor

10. Tekade RK, Youngren-Ortiz SR, Yang H, Haware R, Chougule MB. **Designing hybrid onconase nanocarriers for mesothelioma therapy: a taguchi orthogonal array and multivariate component driven analysis.** ***Molecular Pharmaceutics*****,** 2014, 11, 3671-83. DOI: 10.1021/mp500403b **(IF- 4.78).**

Highlight of this research	**Contribution and Role**
Onconase (ONC) is a member of a ribonuclease superfamily that has cytostatic activity against malignant mesothelioma (MM). The objective of this investigation was to develop bovine serum albumin (BSA)-chitosan based hybrid nanoformulations for the efficient delivery of ONC to MM while minimizing the exposure to normal tissues. Taguchi orthogonal array L9 type design was used to formulate ONC loaded BSA nanocarriers (ONC-ANC) with a mean particle size of 15.78 ± 0.24 nm (ζ = -21.89 ± 0.11 mV). The ONC-ANC surface was hybridized using varying chitosan concentrations ranging between 0.100 and 0.175% w/v to form various ONC loaded hybrid nanocarriers (ONC-HNC). The obtained data set was analyzed by principal component analysis (PCA) and principal component regressions (PCR) to decode the effects of investigated design variables. PCA showed positive correlations between investigated design variables like BSA, ethanol dilution, and total ethanol with particle size and entrapment efficiency (EE) of formulated nanocarriers. PCR showed that the particle size depends on BSA, ethanol dilution, and total ethanol content, while EE was only influenced by BSA content. Further analysis of chitosan and TPP effects used for coating of ONC-ANC by PCR confirmed their positive impacts on the particle size, zeta potential, and prolongation of ONC release compared to uncoated ONC-ANC. PCR analysis of preliminary stability studies showed increase in the particle size and	• **Role:** Postdoctoral research work • Carried out at University of Hawaii, USA • Idea and hypothesis, Project planning and execution

zeta potential at lower pH. However, particle size, zeta potential, and EE of developed HNC were below 63 nm, 31 mV, and 96%, respectively, indicating their stability under subjected buffer conditions. Out of the developed formulations, HNC showed enhanced inhibition of cell viability with lower IC50 against human MM-REN cells compared to ONC and ONC-ANC. This might be attributed to the better cell uptake of HNC, which was confirmed in the cell uptake fluorescence studies. These studies indicated that a developed nanotherapeutic approach might aid in reducing the therapeutic dose of ONC, minimizing adverse effects by limiting the exposure of ONC to normal tissues, and help in the development of new therapeutic forms and routes of administration.	