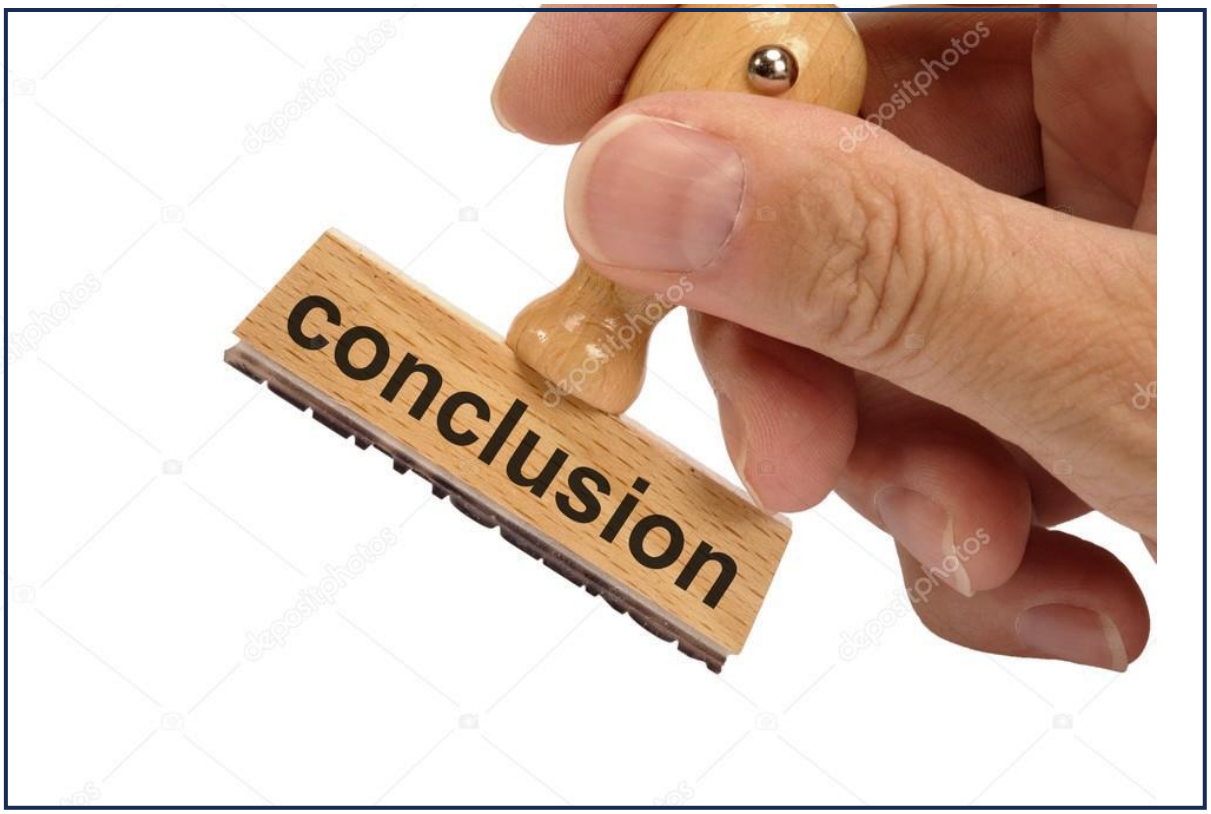
Chapter 6



6.1 Conclusion

In this thesis, a strong emphasis has been placed on the development of LDH and functionalized LDH for tumor treatment through controlled and targeted drug delivery. Among the various combinations of LDH, I focused on Li-Al-based LDH with doxorubicin DOX as the anticancer drug. Machine learning guided me in selecting this specific pair to study controlled drug delivery.

Li-Al-based LDH was synthesized with increasing Al substitution, ensuring better control over particle size (approximately 150 nm) and stability. Higher Al content allowed greater drug intercalation through ion exchange, leading to sustained drug release due to the large lateral dimensions of the LDH and the stronger interaction between LDH and DOX. Spectroscopic analysis, supported by DFT calculations, revealed strong interactions between the components through a significant peak shift, minimal hydrogen-bonded distances, and energy reduction. Embedding negatively charged DOX into the LDH resulted in an overall positively charged species, facilitating cellular uptake through the negatively charged cell membrane. The LDH demonstrated biocompatibility, while DOX-loaded LDH exhibited an impressive 72% cell-killing efficiency within three days, as opposed to a mere 2% with pure DOX. This superior result stemmed from enhanced cellular uptake, as observed through fluorescence imaging. Flow cytometric analysis further confirmed a high rate of apoptosis in cells treated with the LDH-DOX formulation, compared to healthier cells when treated with pure DOX. Apoptotic mechanisms were elucidated through colocalization and the expression of proteins such as caspase-3, p53, and BCL-XL via western blotting. *In vivo* experiments on melanoma-bearing mice indicated a significant reduction in tumor volume when using an injectable hydrogel

formulation, while pure DOX showed only scant tumor reduction. This outcome was attributed to the sustained drug release from the LDH system, further verified through DOX bio-distribution in the bloodstream and vital organs like the liver. The higher concentration of DOX in tumor tissues underscored the efficacy of the hydrogel formulation for enhanced tumor treatment. Histopathological analysis revealed substantial tumor healing in the nanoformulation-treated mice, in contrast to the mild lymphocytic infiltration and small areas of necrosis observed in the pure DOX-treated group. Additionally, healthy liver tissue in formulation-treated mice versus portal tract inflammation and hepatocyte deformation in the pure DOX-treated group suggested that the developed formulation eradicates adverse side effects, which are common in conventional chemotherapy. Immunohistopathological studies further supported the level of protein expression corresponding with tumor healing. Thus, the newly developed LDH-based drug carrier has proven to be highly effective in tumor healing without side effects, addressing the limitations of traditional anticancer therapies.

The hydrophilic, inorganic Li-Al- LDH is elegantly grafted with organic, hydrophobic polyurethane, creating a sophisticated organic-inorganic nanocomposite hybrid with diverse architectural variations. This structural innovation not only enhances thermal stability but also significantly improves mechanical performance, boasting an extraordinary elongation at break of 1230%. This strategic modification allows for a greater quantity of drug to be intercalated into the LDH's interlayer spaces via ion exchange, resulting in a sustained and controlled drug release. Spectroscopic analysis, in tandem with DFT calculations, reveals a pronounced peak shift and reduction in energy, underscoring the strong binding interactions between the components. The minimal hydrogen-bonded distance further accentuates this enhanced interaction. The intercalation of negatively charged doxorubicin into the nanocomposite matrix neutralizes the overall charge of the

formulation, facilitating efficient cellular uptake across negatively charged cell membranes. The nanocomposite itself exhibits excellent biocompatibility, while the drug-embedded nanocomposite showcases a remarkable 95% cancer cell-killing efficiency over three days - a stark contrast to the modest 37% efficiency of pristine doxorubicin. This significant improvement in efficacy is attributed to enhanced cellular uptake, as evidenced by fluorescence imaging. Fluorescence-activated cell sorting analysis further confirms the high levels of apoptotic cell death induced by the formulated nanocomposite, while cells treated with pristine doxorubicin predominantly remain healthy. The apoptotic mechanism behind this effect is illuminated through the co-localization and expression of key proteins such as caspase 3, p53, and Bax, as demonstrated by western blot analysis. Complementing these *in vitro* findings, *in vivo* studies conducted on luciferase-containing melanoma-bearing mice reveal a substantial reduction in tumor volume following treatment with the injectable hydrogel formulation. In contrast, mice treated with pristine doxorubicin exhibit only a modest reduction. This heightened efficacy is attributed to the sustained release of the drug from the nanocomposite system, a conclusion further supported by bio-distribution studies, which indicate higher doxorubicin concentrations in the tumor tissues when using the novel formulation. Histopathological analysis reveals extensive tumor healing in mice treated with the designed nanocomposite formulation, while those treated with pristine doxorubicin show only mild lymphocytic infiltration and limited necrosis. Furthermore, the livers of mice treated with the novel formulation appear healthy post-treatment, in contrast to the inflammation and deformation of hepatocytes observed in pristine doxorubicin-treated mice, underscoring the reduced side effects of the nanocomposite system compared to conventional chemotherapy. Immunohistopathological studies reinforce these findings by corroborating the protein expression levels that align with the observed tumor healing.

In conclusion, this newly developed polyurethane-grafted Li-Al-based nanocomposite drug carrier presents a highly effective treatment strategy for tumors through controlled and targeted therapy of drug over a longer period of time, while circumventing the adverse side effects commonly associated with traditional anticancer therapies. It holds significant promise as a superior alternative in cancer treatment, offering hope for more targeted, efficient, and safer therapeutic options in oncology.

6.2 Plan for Future work:

The detailed set of objectives for a pioneering study cantered on the development of electrochemical biosensors and the creation of cutting-edge materials for biomedical purposes. These goals reflect an innovative approach to both biomedical engineering and material science, aiming to enhance the effectiveness and precision of drug and gene delivery systems.

1. **Variation in Soft and Hard Segments**: A key objective involves the modification of soft and hard segments within polyurethanes, a versatile class of polymers, to create biodegradable versions. This process holds the potential for the synthesis of 3D materials specifically tailored for biomedical applications, enabling enhanced interaction with biological environments and fostering advancements in tissue engineering, implants, and other medical devices.
2. **Polyurethane Nanocomposites**: Another crucial goal focuses on the design and use of polyurethane nanocomposites to develop systems that exhibit superior thermal and mechanical stability. These high-performance materials promise greater durability and resilience, making them ideal candidates for various medical

applications, such as implants, prosthetics, or biosensor devices that must withstand demanding conditions within the body.

3. **Drug Release and Cancer Treatment**: The study also explores the use of polyurethane nanocomposites in drug delivery systems, with a particular emphasis on cancer treatment. By engineering nanocomposites that can regulate and control drug release, the research aims to improve the precision of targeted therapy, minimizing side effects and enhancing the effectiveness of cancer treatments through localized, sustained drug release.
4. **Gene Silencing and Bioactive Delivery**: Looking to the future, the research will expand its focus to include gene silencing techniques, a powerful method for controlling gene expression to treat genetic disorders and diseases like cancer. Coupled with the integration of machine learning and artificial intelligence, this aspect of the study aims to optimize bioactive delivery systems, providing more efficient and personalized therapeutic solutions.

This comprehensive approach not only bridges the gap between material science and medicine but also lays the groundwork for a new era of biomedical innovation, driven by intelligent design and sustainable, high-performance materials.