CHARACTERIZATION AND THERAPEUTIC APPLICATIONS OF POLYMERSOMES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

MAHAM MAQSOOD

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOCHEMISTRY

JULY 2024

Approval of the thesis:

CHARACTERIZATION AND THERAPEUTIC APPLICATIONS OF POLYMERSOMES

submitted by **MAHAM MAQSOOD** in partial fulfillment of the requirements for the degree of **Master of Science** i**n Biochemistry, Middle East Technical University** by,

Prof. Dr. Naci Emre Altun
Dean, **Graduate School of Natural and Applied Sciences** _____

Assoc. Prof. Dr. Özgül Persil Çetinkol
Head of the Department, **Biochemistry** _____

Prof. Dr. Sreeparna Banerjee
Supervisor, **Biochemistry, METU** _____

Prof. Dr. İrem Erel Göktepe
Co-Supervisor, **Chemistry, METU** _____

Examining Committee Members:

Prof. Dr. Dilek Keskin
Engineering Sciences, METU _____

Prof. Dr. Sreeparna Banerjee
Biological Sciences, METU _____

Prof. Dr. İrem Erel Göktepe
Chemistry, METU _____

Prof. Dr. Çağdaş Devrim Son
Biological Sciences, METU _____

Assist. Prof. Aliye Ezgi Güleç Taşkıran
Molecular Biology and Genetics, Başkent University _____

Date: 26.07.2024

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Maham Maqsood

Signature :

ABSTRACT

CHARACTERIZATION AND THERAPEUTIC APPLICATIONS OF POLYMERSOMES

Maqsood, Maham
Master of Science, Biochemistry
Supervisor: Prof. Dr. Sreeparna Banerjee
Co-Supervisor: Prof. Dr. İrem Erel Göktepe

July 2024, 99 pages

Cancer is a heterogeneous disease characterized by uncontrolled cell division and growth. New drug delivery systems are being studied and designed for more efficient targeted delivery of drugs in cancer treatment. This thesis investigates the use of polymersomes, self-assembled structures from polymers, as a targeted drug delivery system for cancer treatment. Here, we explore the potential of glucose oxidase (GOx)-loaded polymersomes to induce cytotoxicity in cancer cells. GOx was incorporated into the polymersomes to utilize its enzymatic activity for generating cytotoxic hydrogen peroxide and intending to act as a model enzyme in creating a safe therapeutic delivery system. The study employed triblock copolymers, namely poly(2-isopropyl-2-oxazoline)-*b*-poly(2-phenyl-2-oxazoline)-*b*-poly(2-isopropyl-2-oxazoline) (PiPOX-*b*-PPhOX-*b*-PiPOX), to fabricate polymersomes using the double emulsion method. Characterization techniques (DLS, TEM, fluorescence imaging, FTIR) revealed size heterogeneity, with aggregates ranging from 200 nm to over 1000 nm. Cell viability assays using SW620 colorectal cancer cells demonstrated good biocompatibility, with both bare and GOx-loaded polymersomes exhibiting minimal cytotoxicity in the medium without glucose. The significant size variation hindered cellular uptake. However, in the presence of a high glucose (4.5

g/L) medium, GOx-loaded polymersomes displayed a significant increase in cell death compared to bare polymersomes, suggesting reactive oxygen species (ROS) mediated cell death via GOx activity. Overall, this study highlights the significant potential of these polymersomes and the opportunity for further optimization. Future research can focus on refining the size distribution, enhancing the loading process, and investigating the cellular uptake mechanisms to boost the therapeutic efficacy of these constructs.

Keywords: Polymersome, Drug Delivery Systems, Colorectal Cancer, Cell Viability, Glucose Oxidase

ÖZ

POLİMERZOMLARIN KARAKTERİZASYONU VE TERAPÖTİK UYGULAMALARI

Maqsood, Maham
Yüksek Lisans, Biyokimya
Tez Yöneticisi: Prof. Dr. Sreeparna Banerjee
Ortak Tez Yöneticisi: Prof. Dr. İrem Erel Göktepe

Temmuz 2024, 99 sayfa

Kanser, kontrolsüz hücre bölünmesi ve büyümesi ile tanımlanan heterojen bir hastalıktır. Kanser tedavisinde ilaçların daha etkili bir şekilde hedefe yönelik olarak verilmesi için yeni ilaç iletim sistemlerinin tasarlanması üzerine çalışmalar yapılmaktadır. Bu tez çalışması, polimerlerin kendiliğinden yapılanması ile oluşan polimerzomların kanser tedavisinde hedefe yönelik bir ilaç dağıtım sistemi olarak kullanımını araştırmaktadır. Glukoz oksidaz (GOx) yüklü polimerzomların kanser hücrelerinde sitotoksisiteyi indükleme potansiyelini incelenmiştir. GOx, enzimatik aktivitesini kullanarak sitotoksik hidrojen peroksit üretmek ve güvenli bir terapötik dağıtım sistemi oluşturmak amacıyla model bir enzim olarak polimerzomlara dahil edilmiştir. Çalışmada, çift emülsiyon yöntemi kullanılarak polimerzomlara dahil edilmiştir. Polimerzomlar, poli(2-izopropil-2-oksazolin)-*b*-poli(2-fenil-2-oksazoline)-*b*-poli(2-izopropil-2-oksazoline) (PiPOX-*b*-PPhOX-*b*-PiPOX) kullanarak çift emülsiyon yöntemiyle hazırlanmıştır. Karakterizasyon teknikleri (DLS, TEM, floresan görüntüleme, FTIR) 200 nm ile 1000 nm arasında değişen agregatlar olduğunu göstermiş ve boyut heterojenliğini ortaya koymuştur. SW620 kolorektal kanser hücreleri kullanılarak yapılan hücre canlılığı deneyleri, hem içi boş hem de GOx yüklü polimerzomların glukoz içermeyen ortamda minimum

sitotoksisite sergilemesiyle iyi biyouyumluluk göstermiştir. Polimerzomlar arasındaki önemli boyut farklılığı hücresel alımı engellemiştir. Bununla birlikte, yüksek glukozlu (4,5 g/L) bir ortam varlığında, GOx yüklü polimerzomlar, içi boş polimerzomlara kıyasla hücre ölümünde önemli bir artış sağlamıştır. Bu da, GOx aktivitesi yoluyla reaktif oksijen türleri (ROS) aracılı hücre ölümüne işaret etmektedir. Genel olarak, bu çalışma bu polimerzomların önemli potansiyelini ve daha fazla optimizasyon fırsatını vurgulamaktadır. Gelecekteki araştırmalar, bu yapıların terapötik etkinliğini artırmak için boyut dağılımını iyileştirmeye, yükleme sürecini geliştirmeye ve hücresel alım mekanizmalarını araştırmaya odaklanabilir.

Anahtar Kelimeler: Polimerzom, İlaç Taşıma Sistemleri, Kolorektal Kanser, Hücre Canlılığı, Glukoz Oksidaz

To my beloved parents

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor, Prof. Dr. Sreeparna Banerjee, and my co-supervisor, Prof. Dr. Irem Erel Göktepe, for giving me the opportunity to work in their labs. Their guidance, insights, and valuable criticism have been instrumental throughout my research. I would also like to thank my jury members Prof. Dr. Dilek Keskin, Prof. Dr. Çağdaş Devrim Son, and Assist. Prof. Aliye Ezgi Güleç Taşkıran for their suggestions, comments, and guidance for my thesis.

I would like to extend my heartfelt thanks to Cemre Alemdar for the continuous assistance and help she provided me during my research. I am also grateful to my lab mates Dilara Gündoğdu, Ayşe Güren, İpek Terzioğlu, Doğa Postacı, and Tuna Gökçe for their kindness and help. I would like to especially acknowledge Çağrı Turan, who synthesized the triblock copolymer utilized in this study and optimized the conditions for polymersome preparation. The polymer synthesis and determination of the procedure for the preparation of GOx-containing polymersomes were first investigated as part of the project, which was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Grant Number: 219Z329). I would also like to thank Esin Gülce Seza and İsmail Güderer for the guidance and support they provided during my work at the biology department. Special thanks to Aydan Torun for being the kindest soul and for helping me out in settling in during the early days of my master's study. I appreciate the support and camaraderie of my colleagues Nazlı Şevval Menemenli, Dr. Hepşen Hazal Hüsnügil, and Özün Özcan, who made my time at the Banerjee lab memorable.

I would like to thank my dear husband, Muhammad Taha, for his endless support and love, for which I am forever grateful.

I acknowledge Yurtdışı Türkler ve Akraba Topluluklar Başkanlığı (YTB) for the scholarship award that funded my studies at METU.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS

AFM	Atomic Force Microscopy
CC	Cytochrome C
CMC	Critical Micelle Concentration
CROP	Cationic Ring Opening Polymerization
Cryo-TEM	Cryo-Transmission Electron Microscopy
CSCs	Cancer Stem Cells
DCM	Dichloromethane
DDS	Drug Delivery Systems
DHA	Docosahexaenoic Acid
DHF	Dihydrofolate
DLS	Dynamic Light Scattering
DOX	Doxorubicin
DTX	Docetaxel
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial to Mesenchymal Transition
EPA	Eicosapentaenoic Acid
EpCAM	Epithelial Cell Adhesion Molecule
FITC	Fluorescein Isothiocyanate

FTIR	Fourier-Transform Infrared spectroscopy
GOx	Glucose Oxidase
iPOX	2-Isopropyl-2-Oxazoline
MALS	Multi-Angle Light Scattering
MDR	Multi-Drug Resistance
MLV	Multilamellar Vesicle
MMP-9	Matrix Metalloproteinase 9
MTT	[3-(4, 5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide]
NIRF	Near-Infrared Fluorescence
NMR	Nuclear Magnetic Resonance
PAA-*b*-PS	Polyacrylic Acid-*b*-Polystyrene
PAOXs	Poly(2-alkyl-2-oxazoline)s
PBLG-*b*-HYA	Poly (g-benzyl L-glutamate)-*b*-Hyaluronan
PDI	Polydispersity Index
PDMS-*b*-PMOXA	Poly(dimethylsiloxane)-*b*-poly(methyloxazoline)
PEG	Poly(ethylene glycol)
PEG-*b*-PCL	Poly(ethylene glycol)-*b*-poly(ε-caprolactone)
PEG-*b*-PS	Poly(ethylene glycol)-*b*-polystyrene

PEO-*b*-PBD	Polyethylene oxide-*b*-polybutadiene
PEO-*b*-PDLLA	Polyethylene oxide-*b*-poly(D,L-lactide)
PEO-*b*-PS	Polyethylene oxide-*b*-polystyrene
PEtOx	Poly(2-ethyl-2-oxazoline)
PhOX	2-Phenyl-2-Oxazoline
PISA	Polymerization-Induced Self-Assembly
PLA	Polylactic Acid
PLGA	Polylactide-co-glycolide
PMPC-*b*-PDPA	Poly(2-methacryloyloxy)ethylphosphorylcholine-*b*-poly(2(diisopropylamino)- ethyl methacrylate
Ps	Polymersomes
PTMC-*b*-PGA	Poly(trimethylene carbonate)-*b*-poly(L-glutamic acid)
PVA	Polyvinyl Alcohol
RAFT	Reversible Addition Fragmentation chain Transfer
RES	Reticuloendothelial System
SEM	Scanning Electron Microscopy
SPECT/CT	Single-Photon Emission Computed Tomography/Computed
SPIONs	Superparamagnetic Iron Oxide Nanoparticles

TEM	Transmission Electron Microscopy
Tetrac	Tetraiodothyroacetic acid
THF	Tetrahydrofolate
TKI	Tyrosine Kinase Inhibitors
TNF- α	Tumor Necrosis Factor-α

CHAPTER 1

INTRODUCTION

1.1 Overview of Cancer as a Global Health Issue

Cancer is one of the most elusive and complex diseases of our time. Characterized by uncontrolled growth of cells and proliferation, it is a global health concern in developed as well as in developing countries and is becoming a leading cause of mortality (Kawahara et al., 2010). It is often precepted as a disease that occurs as a result of lifestyle choices (Kawahara et al., 2011). The need for cancer prevention is apparent, especially by addressing risk factors like the use of tobacco, obesity, infections, environmental influence, etc. (Braithwaite et al., 2012).

Cancer treatment challenges are multifaceted, including drug resistance, tumor heterogeneity, and acquired resistance; nonetheless, there has been significant advancements in cancer therapy, particularly in the development of targeted and immunotherapies (Nagaraju & Kamal, 2020; Phelps & Sparreboom, 2014). These advances have no doubt improved survival rates and raised the quality of life for cancer patients, but the success is limited, often specific to tumor types as well as patient characteristics. Thus, there is a need for further improvements in outcome measures and discoveries of new treatment modalities (Phelps & Sparreboom, 2014). The future of cancer treatment is greatly associated with the development of personalized therapeutic approaches, which are able to overcome the challenges and optimize treatments (Jianqing & Ping, 2023).

1.1.1 Cancer Therapeutics and Challenges

Cancer therapeutics represent a multifaceted approach to fight malignancies and integrate a diverse array of treatment methodologies tailored to tumor characteristics

and, sometimes, to individual patient profiles (Thenuwara et al., 2023). Each therapy carries unique advantages and challenges. The landscape of cancer treatment is continuously changing, with traditional methods being replaced by new innovations. Healthcare professionals navigate treatment decisions by understanding the unique mechanisms of action and clinical applications of each therapy.

1.1.2 Current Challenges in Cancer Therapeutics

Cancer therapeutics face complex challenges from intrinsic to environmental influences that hinder treatment efficacy and significantly affect patient outcomes (Wu et al., 2018). Cancer cells are adaptive and highly efficient, and can evade the effects of therapeutic agents, making them ineffective overtime. Moreover, the intricacies of tumor microenvironments that is characterized by hypoxia, nutrient deprivation, and immunosuppression can further complicate the treatment strategies (Augustin et al., 2020). This section aims to showcase some of the obstacles faced in the pursuit of effective cancer therapeutics.

1.1.2.1 Drug resistance

The challenge of drug resistance in cancer therapeutics is one of the most prevalent and significant obstacles, with both intrinsic and acquired resistance being common (Nagaraju & Kamal, 2020). Reduced drug uptake, increased energy-dependent efflux, and changes in cells that affect the ability of drugs to kill cancer cells are some of the mechanisms involved in drug resistance (Szakács et al., 2006). These are being tackled by the development of novel therapeutic compounds that can overcome resistance by improved solubility, bioavailability in the tumor microenvironment, as well as enhanced specificity (Nagaraju & Kamal, 2020; Ward et al., 2021). There is a need to develop a multi-faceted approach, such as the development of targeted therapies and the identification of prognostic biomarkers that can reliably report on the efficacy of the drugs (S. K. Upadhyay et al., 2017).

1.1.2.2 Toxicity to Healthy Tissues

Toxicity is a major concern and is highly associated with current cancer therapy treatments (Schuchter et al., 1992). The more proliferative a normal tissue is, such as the bone marrow which has high cell turnover, the more it is susceptible to toxicity (Schiff et al., 2009). Oxidative stress, a key mechanism of toxicity, is particularly damaging to the heart and brain (Y. Chen et al., 2007). Certain inhibitors can also have adverse effects such as Tyrosine Kinase Inhibitors (TKI) that targets growth factor receptors, but because of its low specificity, it can also lead to toxicity in normal non-transformed cells (Shyam Sunder et al., 2023).

The toxicity of cell therapy which includes adoptive cell therapies, is a major concern that is being addressed and studied by scientists to manage it efficiently (Jin et al., 2021). This problem is addressed through the development of toxicity antagonists, which are agents that regulate normal tissue response and interfere with the toxicity mechanisms by acting as modulators of tissue repair, antagonists of reactive species, growth factors, and anti-inflammatory agents (Trotti, 1997). Cytoprotective agents, which include chemical compounds from natural resources that protect cells, are also being explored to reduce treatment-related toxicity without compromising the efficacy (Diwanay et al., 2004). Moreover, marine-derived lipids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have exhibited potential in selectively sensitizing tumors to chemotherapy by enhancing the cytotoxicity of anticancer drugs, thereby reducing the toxicity to non-tumor tissues (Hajjaji & Bougnoux, 2013).

1.1.2.3 Tumor Heterogeneity

Tumor heterogeneity is another significant challenge in cancer therapeutics. Macroevolutionary adaptations and the tumor microenvironment play major roles in driving tumor evolution and metastasis, which highlights the need for new approaches to drug development and clinical trial design (McGranahan & Swanton,

2017). In this regard, single-cell RNA sequencing and next-generation sequencing technologies carry potential in dissecting the diverse cellular populations of tumors, which can enhance targeted combination therapies and inform clinical trial enrollment criteria (Koltai, 2017; Levitin et al., 2018). Several studies have highlighted the importance of personalized strategies in targeting tumorigenic cell populations, along with advances in systems biology that can help in understanding of cancer progression and drug response (Fedele et al., 2014).

1.1.2.4 Immune Evasion

The cancer immune landscape entails the expression of neoantigens in tumor cells as well as the release of antigens when the transformed cells die. These antigens are processed and presented, leading to the infiltration of $CD8^+$ T cells into tumors in the tumor microenvironment (Tang et al., 2020). In this manner, the immune system can effectively eliminate rogue transformed cells; however, very often cancer cells develop several strategies to evade and suppress the immune system, allowing for the cancer cells to escape from $CD8^+$ lymphocyte mediated elimination. Additionally, T cell exhaustion is an important factor whereby the cells become hyporesponsive, express high levels of inhibitory receptors such as PDI, which prevents the T cell from recognizing cancer cells and prevents their elimination (Jiang et al., 2015).

Cancer immune evasion poses a major hurdle in the application and design of effective anticancer therapeutic strategies (Zagozdzon et al., 2022). A range of factors, like metastasis, the ability of tumors to exploit immunological processes such as enhancing the regulatory T cell function, recruitment of myeloid derived suppressor cells, and tumor heterogeneity, among others, collectively facilitate immune evasion (Vinay et al., 2015). The dynamic co-evolution of tumors and the immune systems further underscores the complexity of this challenge (George & Levine, 2021). Despite these obstacles, research into potential therapeutic strategies continues, such as promoting specific immune responses and inhibiting immune-

suppressive mediators (Vinay et al., 2015). Checkpoint inhibitors are crucial in cancer immunotherapy, targeting proteins like CTLA-4, PD-1, and PD-L1 to restore immune response against tumor cells (Shiravand et al., 2022). They work by blocking the immune evasion mechanisms utilized by cancer cells and allow the immune system to combat cancer effectively. There is also the employment of adoptive T-cell therapy, vaccine development, and combination therapies to overcome immune evasion (Tang et al., 2020).

1.1.2.5 Limited Efficacy in Late-Stage Cancer

The limited effectiveness in advanced stage of cancer is a major problem, with chemotherapy often falling short due to its limited potency and serious side effects (Kakde Deepak, 2011). Targeted therapies, such as ligand-targeted therapy, have shown promise in addressing this challenge, but still face barriers like drug resistance and high tumor interstitial fluid pressure (Wu Han-Chung, 2006). While immunotherapy is widely hailed as a breakthrough, it also faces issues in predicting treatment efficacy and patient response (Ventola, 2017). Furthermore, chemotherapy-enhanced inflammation may contribute to therapy failure and metastasis (Vyas et al., 2014).

1.1.2.6 Accessibility and Cost

The high cost of cancer care presents a major challenge to market access. Cancer medication cost is the highest monthly member expenditure in the US. This issue is worse in low and middle-income countries where access to therapeutics is limited due to financial constraints and regulatory delays (Ruff et al., 2016). Novel paradigms are needed to ensure the affordability of anticancer drugs, and there is a need for collaboration between key stakeholders, including pharmaceutical companies, as well as health authorities who have the power to increase the accessibility of drugs (Ruff et al., 2016; Sleijfer & Verweij, 2016).

1.2 New Therapies in Cancer

Recent developments in cancer therapies aim towards targeted treatments that can minimize damage to normal cells (Kim, 2003). These types of treatments are driven by a greater understanding of tumor biology and the development of anticancer drugs that can specifically target cancer cells, as normal cells often fall prey due to imprecise targeting (Ramaswami et al., 2013). Novel approaches, like gene therapy, antiangiogenic therapy, and monoclonal antibodies, are being studied to enhance efficacy and reduce toxicity (Joensuu, 2000). In addition, therapies like stem cell therapy, ablation therapy, and the use of natural antioxidants are showing promise in regenerating tissues, minimizing invasiveness, and neutralizing harmful effects (Debela et al., 2021).

In this regard, personalized and precision medicine in cancer therapy is one of the rapidly evolving fields within cancer therapy that considers the genetic, environmental, and lifestyle factors of each patient (Hoeben et al., 2021). This approach not only targets cancer cell proliferation but also considers the patient's profile like the immune system, physiological functions, and metabolic activity, among others (Bing & Shing CHO, 2017). It makes use of the information from lab tests to develop a customized care plan, which can help in improving diagnosis and treatment (Saranath & Khanna, 2015). Advances in precision medicine have also led to the discovery of new biomarkers and therapeutic agents, allowing for personalized treatment plans (Matchett et al., 2017).

Nanotechnology, which includes the use of polymersomes, is another promising approach in cancer therapy. Polymersomes can deliver anticancer drugs to solid tumors, and have the potential for combination with other treatment methods (Kuperkar et al., 2022). Additionally, the development of hybrid materials, such as carbon nanostructures combined with polymers are also a means of enhancing the efficacy of chemo- and radiotherapies (Cirillo et al., 2019). Polymer-based materials are utilized in image-guided cancer therapy as well, which can potentially

revolutionize the field by enabling precise drug delivery to targeted sites (Sun et al., 2022).

1.3 Drug Delivery Systems

Drug delivery systems (DDS) control the movement of drugs in the body to optimize their therapeutic features. These systems have evolved over time, and the recent advancements include the use of polymer nanoparticles that are also biodegradable, and delivery through different routes (Tiwari et al., 2012). The aim of using DDS is to improve disease outcomes and specific targeting of drug receptors without undesired interactions (Bassyouni et al., 2015)

Traditional drug administration methods, such as oral and injectable routes, have limitations that can reduce their effectiveness, sometimes within specific patient populations. These limitations include the need for rapid response, systemic effects rather than local, aggravated physical symptoms like vomiting, etc (Benson & Prankerd, 1997). The reliance on such methods has been mostly due to technological constraints. However, with novel drug delivery systems that facilitate controlled administration, improved safety and efficacy have been observed (Michaels, 1974).

The advancement in Drug Delivery Systems (DDS) has significantly impacted cancer research (Bae & Park, 2020). These advancements have enabled targeted and effective delivery of drugs, which improves treatment outcomes and reduces side effects for patients. Moreover, the use of polymeric and magnetic nanoparticles in these systems has shown promise in enhancing the effectiveness of cancer treatments. The development of hybrid nanocarriers has also introduced new therapeutic and diagnostic applications in cancer research and targeted tumor therapy (G. Liu et al., 2021). The nanoparticle drug delivery systems, in particular, have been studied for their potential in reversing multidrug resistance in cancer (HUANG et al., 2016). Novel drug delivery systems, such as the controlled release microchips and polymer-drug conjugates, have helped enhance the clinical application of cancer

therapeutics (Prasanna N., 2013). However, there remain challenges like physical barriers, tumor heterogeneity, drug resistance, etc. in spite of these advancements, emphasizing the need for a multidisciplinary approach in cancer therapy (Z.-R. Lu & Qiao, 2018).

1.3.1 Principle of Drug Delivery Systems

Drug delivery systems (DDS) are used in cancer therapeutics with the aim to maximize the efficacy of drugs and reduce side effects. Delivery in a controlled and targeted manner is necessary to ensure the desired therapeutic effect is achieved. These systems for drug delivery are specifically designed to target cancer cells or tumor tissues, allowing for the higher accumulation of the drug at the site of action, which minimizes drug loss and increases drug concentration at the tumor site (Minko et al., 2004).

DDS can evade the immune system through various mechanisms. Poly(ethylene) glycol (PEG) has been shown to play an important role in modifying the composition of proteins on nanomaterials, allowing them to evade immune detection (Butcher et al., 2016). There has been development and use of nano- and micro- particles in cancer immunotherapy and vaccines, which can train immune cells to recognize and eliminate cancer cells (Batty et al., 2019). Studies have reported DDS that prevents immune activation by using particles that can travel through bloodstream without being phagocytosed by macrophages (Marshall, 2001). The Reticuloendothelial System (RES) contains cells from monocytes that can phagocytose foreign particles. Various strategies have been developed to bypass RES, such as the use of pre-blocking RES receptors using HA-coated liposomes before administering the DDS, thereby weaking RES clearance and enhancing tumor targeting efficiency (F. Liu et al., 2018).

These systems can also provide controlled release over an extended time period, which maintains the drug levels in the body for a longer duration and reduces the frequency of dosing while improving patient compliance (Adepu & Ramakrishna,

2021). Scientists are designing these systems to utilize biocompatible and biodegradable materials, such as polymers, lipids, or nanoparticles, to encapsulate and deliver the drug (Kumari et al., 2010). Such materials are used for their ability to ensure the sustained release of drugs, and are also able to gradually degrade in the body over time (Kumari et al., 2010). There are also DDS that incorporate protective coatings or encapsulation methods to shield the encapsulated drug from degradation in the harsh physiological environment (J. Liu et al., 2017). Poor drug solubility and bioavailability is a challenge that a DDS can overcome by improving drug solubility and enhancing its absorption and distribution in the body (Kesharwani et al., 2023). These mechanisms are helpful in improving the effectiveness of these types of cancer therapeutics.

1.3.2 Targeted drug Delivery

DDS are able to perform targeted delivery of therapeutic agents to tumor sites while minimizing systemic toxicity. In this regard, these systems tend to take advantage of the unique characteristics and physiology of the tumors, such as their disordered structure and leaky vasculature, to deliver anticancer drugs with diminished side effects (AlSawaftah et al., 2021). For instance, one of the most well-studied delivery systems in cancer therapy are liposomes, and their specificity and bioavailability can be improved through surface functionalization and stimuli-mediated drug release that takes into account and manipulates the tumor physiology (AlSawaftah et al., 2021). DDS can also be designed to deliver combination therapies for cancer by targeting synergistic therapeutic agents to the tumor site and enhancing their activity (Eldar-Boock et al., 2013).

Light-responsive DDS are photo-responsive and can release drugs when light at a specific wavelength is used at the tumor site; such systems can reduce systemic toxicity (H. Chen & Zhao, 2018). Additionally, stimuli-responsive nanoscale DDS that can release drugs in response to specific stimuli, such as pH, temperature, and

redox potential, have also been developed to improve efficacy and reduce side effects of traditional anticancer drugs (L. Li et al., 2019).

1.3.3 Advantages and Disadvantages of Drug Delivery Systems

DDS in cancer therapeutics has several advantages, such as the ability to deliver hard-to-formulate drugs that include sparingly water-soluble active drugs, decrease toxicity, and localize therapy to the site of action (Martini & Ciocca, 2003). Nanomaterial-based systems, such as drug nanoparticles, have more advantages in terms of ease of preparation, high efficiency, low toxicity, and ability to target tumors exclusively (G. Liu et al., 2021). Nanocarriers, like micelles and liposomes, can improve the pharmacological properties of drugs and have also shown clinical success (Cukierman & Khan, 2010). The multiple stimuli-responsive drug delivery systems offer the ability to overcome physiological and pathological barriers and co-deliver agents (Jia et al., 2021).

Like other systems, DDS also have several disadvantages. Some DDS can still have toxic side effects, but those could be to a lesser extent than conventional drug treatments (Milewska et al., 2021). There is also the risk of sudden release or "dose-dumping" of drugs from certain delivery systems, like polymeric nanoparticles, which can lead to adverse reactions or ineffective treatment (Sanadgol & Wackerlig, 2020). Some DDS have limited capacity to load or deliver therapeutic agents, which hinders their use in therapeutic applications (Elumalai et al., 2024). The retention time is another challenge, and in one study, it has been observed that phagocytes absorbed microparticles smaller than 10 μm and caused them to seep out of inflamed joints in mice (Pradal et al., 2016).

1.3.4 Physiological Parameters for Drug Delivery in Cancer Models

A range of physiological parameters influence drug delivery in cancer models. There is a crosstalk between tumor properties, drug transport, and cellular uptake, and

researchers are using computational models to define such relationships and optimize drug delivery systems (Zhan et al., 2018). For the cancer models and associated risk assessment, the use of pharmacokinetics in predicting the doses of toxic chemicals delivered at target tissues is necessary (Andersen et al., 1993). The physical and physiological properties of tumors, such as accumulated solid stress of malignant cells that compresses blood vessels, nonfunctional lymphatics, etc., create barriers to drug delivery and thus require a more strategic drug development process (Chauhan et al., 2011). The drug transport limitations due to the tumor-associated changes in the vasculature and extracellular matrix have prompted the use of tissue-engineered tumor models to study these processes better (Seo et al., 2014). Thus, it is very important to consider such parameters for the design and optimization of DDS for cancer treatment.

1.3.5 Overcoming Drug Resistance in Cancer

Drug reistance in cancer is a complex and multifaceted issue with various mechanisms at play. The mechanisms enabling resistance include DNA damage repair, cell death inhibition, drug efflux, target alteration, the epithelial-to-mesenchymal transition (EMT), and epigenetic modifications (Housman et al., 2014). There is a need to understand the genetic and microenvironmental factors that modulate these outcomes, and a need to develop novel approaches to overcome drug resistance (Haider et al., 2020; Lei et al., 2023). Identifying and targeting specific pathways and mechanisms of resistance, with a focus on small molecule therapeutics and combination therapies, are important factors to be considered in overcoming drug resistance in cancer (Ferrarelli, 2015).

1.3.6 Nanosystems

New developments in DDS focus on improving patient compliance and safety. These advancements include the use of smart polymers, nanotechnology, and the

incorporation of nanoparticles into microdevices to enhance release and targeting of drugs (Park, 2014). The pharmaceutical industry has been revolutionized by these types of innovations, which have led to the creation of new formulations and improved treatment outcomes. The creation of tailored delivery systems with synthetic nanometer-sized particles is one of the key milestones, that allow for the delivery of more complex therapeutic agents (Kiparissides & Kammona, 2008). Nanosystems in drug delivery for cancer therapy have shown significant potential in improving treatment efficacy and reducing toxicity. These nanoparticles have the ability to deliver high drug concentrations to cancer cells while reducing damage to normal tissue (Dadwal, 2014).

However, nanodelivery faces several challenges as well; for instance the blood-brain barrier that limits the effectiveness of these systems (Nyström & Fadeel, 2012), as well as safety concerns, including potential side effects (Khare et al., 2015). While promising, nanodelivery systems can also have intrinsic toxicological problems due to their size and shape, as well as physical interaction with biological membranes, as well as long-term tissue accumulation (M. Bimbo et al., 2012). There are also complications due to the need for careful design and engineering, development of reproducible manufacturing process, and thorough safety and efficacy assessments (Desai, 2012).

All in all, nanosystems in drug delivery have significant implications for research. Micro- and nanosystems are already successfully employed for drug administration and carry the potential for on-demand triggering of molecular interactions and diffusion-controlled distribution methods and delivery devices (LaVan et al., 2003). Along with improved bioavailability and controlled delivery, the revolutionary changes brought by these systems are seen in the research and treatment of cardiovascular defects, autoimmune diseases, and cancer (Simonazzi et al., 2018).

1.3.7 Polymers in Nanosystems

Polymers are crucial in nanotechnology and its applications due to their nanoscale length ranges and morphologies, which makes them ideal for nanostructure fabrication (H. Li & S. Huck, 2002). They can be used with versatile strategies and can be functionalized with different materials for applications in nanophotonics and nanobiosystems (Nguyen et al., 2014). Moreover, they have tunable properties and can serve as templates for fabrication due to their different morphology and sizes, and ease of extractability after use (T. Liu et al., 2003).

1.3.7.1 Copolymers

Copolymers are a type of polymer made up of at least two different types of monomers. There are several different types of copolymers depending on how the repeating units are arranged in the polymer chain. Random copolymers have two or more monomers that can exist in any order, and alternating copolymers usually have monomers arranged in an alternate manner within a chain. Graft copolymers are another type that have chains of one polymer attached to chains of another. In this thesis work, block copolymers, which contain a combination of blocks of monomer units, are of interest.

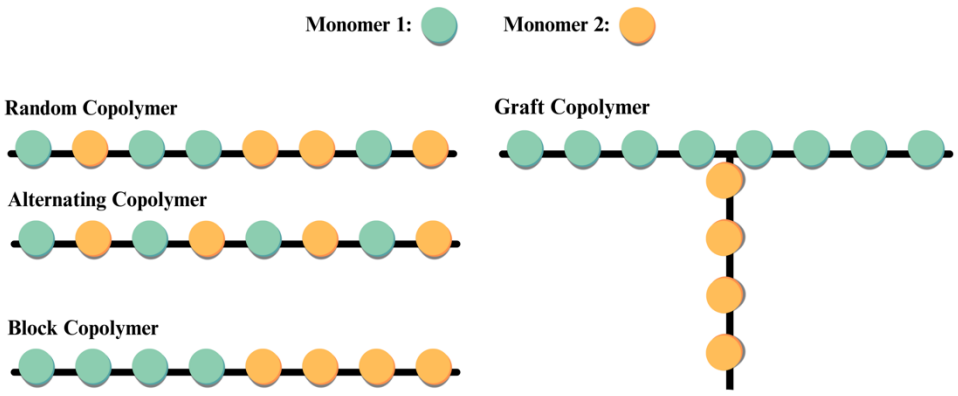


Figure 1.1. Illustration of various types of copolymers with their characteristic composition

They exhibit unique characteristics and properties due to their molecular structure, including amphiphilic features in solution and microdomain formation in solid state (Riess & Hurtrez, 1996). These materials have a range of applications. The development of block copolymers have significantly advanced polymer chemistry, allowing for the creation of a diverse range of properties and applications in various fields (Shi et al., 2021).

Self-assembly of Polymers

The self-assembly of polymers is a versatile process that can be used to create a wide range of functional materials (Vikulina & Volodkin, 2019). The self-assembly features of amphiphilic copolymers, as well as biomolecules, are used to develop materials like micelles, nanoparticles, and polymersomes.

Self-assembly of Block Copolymers

Self-assembly of block copolymers is a complex process that can lead to the formation of a variety of ordered structures (Abetz, 2020). Several techniques have been used to direct and control this process such as the use of lithographically defined, chemically nanopattered substrates that have the ability to reduce critical dimension variation and generate unique device-oriented patterns (Craig & Nealey, 2007). Moreover, the self-assembly of organic-inorganic block copolymers have been used to create nanostructures with specific properties, such as a hard silica mask (Hirai et al., 2009). This phenomenon has also been demonstrated by the block copolymer blend of fluorocarbon end-functionalized polybutylmethacrylate and polystyrene into materials which are optically transparent with well-defined lamellar nanostructured sturctures (Shen & Hogen-Esch, 2008).

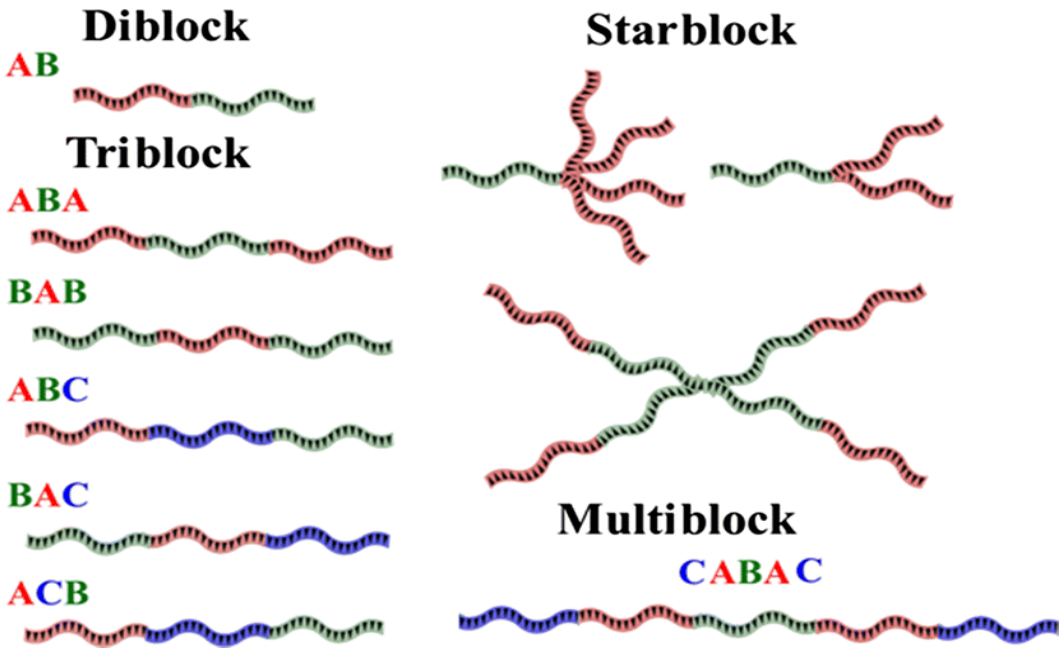


Figure 1.2. Different types of block copolymers and their arrangement (Trotti, 1997)

During the self-assembly of block copolymers, local interactions within a system transform the disordered systems into an ordered one. Amphiphilic block copolymers contain two blocks that have hydrophobic and hydrophilic regions. These varying blocks tend to behave contrastingly after dissolution in a solvent. This can be elucidated by the behavior which the diblock copolymers exhibit when dissolved in an aqueous environment. The hydrophilic blocks are attracted to water, whereas the hydrophobic blocks aggregate together where the polymer interaction is stronger within itself than the polymer-water. This self-assembly is followed by the formation of aggregates with different sizes and shapes.

Triblock Copolymers

For triblock copolymers, self-assembly has also been studied, and various factors like initial solvent composition and polymer concentration have been found to affect the formation of vesicles and micelles. End groups, chain structure, and

stereocomplexation affect the morphology of particles and can be manipulated to further tune nanostructures (Socka et al., 2018). Qi et al. synthesized amphiphilic triblock copolymers, poly(ethylene glycol)-*block*-poly(ε-caprolactone)-*block*-poly(allyl glycidyl ether) (mPEG-*b*-PCL-*b*-PAGE), and observed the formation of micelles that could be utilized in nanomedicine (Qi et al., 2017). Zhang et al. investigated the self-assembly of nucleobase-functionalzied ABC triblock copolymers, which formed into well-defined lamellar microphase-separated morphologies (Zhang et al., 2014).

The self-assembly of triblock copolymers can also lead to the formation of a lattice and gelation in the process known as polymerization-induced self-assembly (PISA) (Yamanaka et al., 2023). Like others, the specific self-assembly behavior of triblock copolymers also depends on the nature of polymer blocks and conditions which can be manipulated to design and tailor the properties of different materials.

1.4 Polymersomes: Characterization, properties, and applications in cancer research

Polymersomes, self-assembled vesicles made from amphiphilic block copolymers, have shown promise in various applications and can be used as drug carriers, with the potential of releasing drugs in a controlled manner in response to specific stimuli (Liao et al., 2012). The unique properties of polymersomes, for instance, high thermal and mechanical stability, make them suitable for a range of biomedical applications (Sachsenhofer et al., 2007). Furthermore, they have been extensively researched for their interfacial properties and have been found to be effective as aqueous lubricants (Bartenstein et al., 2018). Polymersomes have a lot of potential for use in therapeutic industry as their chemical structure, along with the formulation methods, have made them a versatile and promising drug carrier (Kotha et al., 2023a).

These are the type of nanoparticles that can encapsulate drugs within the hydrophilic core or the hydrophobic drugs in the middle part of the bilayer. The sizes of polymersomes can range from less than 100 nm to several micrometers. Their size can also be controlled by altering the relative hydrodynamic volume fraction ratio between hydrophilic and hydrophobic blocks in the copolymer and by changing the fabrication methods (Y. Lee et al., 2006). Yildiz et al. proposed that polymersome formation is governed by the two solvents' limited mutual solubility and the simultaneous diffusion of solvent and water (Yildiz et al., 2007).

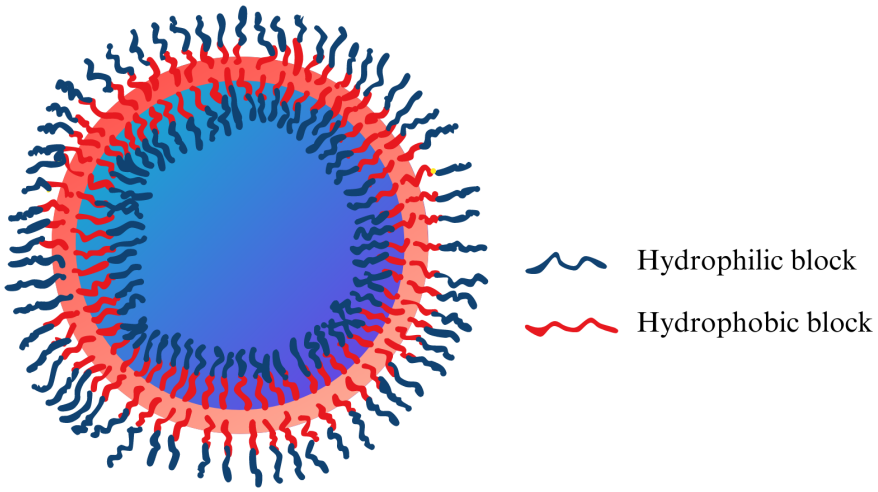


Figure 1.3. Structure of a polymersome made from hydrophilic and hydrophobic polymer blocks

1.4.1 Characterization of Polymersomes

Polymersomes have similar morphology to biological substances like liposomes and cell membranes, and their shape can be characterized using scattering techniques such as electron microscopy, dynamic light scattering (DLS), multi-angle light scattering (MALS), which can distinguish between different types of shapes like

spheres, discs, etc. (Abdelmohsen et al., 2016). The structural and mechanical characteristics of these nanostructures can also be controlled by manipulating their morphology which in turn affects their properties and behavior (Chang et al., 2014). Because of these features, polymersomes have gained attention for their potential use in therapeutics.

1.4.1.1 Structural Composition and Morphology

Polymersomes, as multi-faceted vesicular structures, showcase a wide range of structural composition and morphologies. Understanding the structural characteristics enables researchers to curate and tailor them as per specific therapeutic requirements. A few of their crucial characteristics are described below.

1.4.1.1.1 Lipid Composition

Polymersomes are usually analogous to bilayer structures in mammalian cells as they are composed of amphiphilic block copolymers that have hydrophilic and hydrophobic regions that can self-assemble into bilayers resembling lipid membranes (Rideau et al., 2018). The physicochemical properties of polymersomes including stability, permeability, and responsiveness, are defined by the type of block copolymer used in their design (Kuperkar et al., 2022). Some of the commonly used diblock copolymers are polyethylene oxide-*block*-polystyrene (PEO-*b*-PS), polyethylene oxide-*block*-poly (1,2-butadiene) (PEO-*b*-PBD), polyacrylic acid-*block*-polystyrene (PAA-*b*-PS), polyethylene oxide-*block*-poly(D,L-lactide) (PEO-*b*-PDLLA), and poly(2-methacryloyloxy)ethylphosphorylcholine-*block*-poly(2(diisopropylamino)- ethyl methacrylate (PMPC-*b*-PDPA) (C. K. Wong et al., 2023).

1.4.1.1.2 Size Distribution

Polymersomes exhibit adjustable size distribution ranging from nanometers to micrometers. The size of polymersomes can be affected across the various stages of their preparation (Bleul et al., 2015). The parameters involved in regulating aggregate size include the type of the copolymer, concentration of the copolymer, temperature, nature of the solvent, concentration of water, etc. (Lim Soo & Eisenberg, 2004). Various methods employed in this regard include solvent evaporation, nanoprecipitation, and microfluidic approaches (Bleul et al., 2015). Size control for polymersomes has also been reported by the addition of different-sized magnetic nanoparticles in the polymersome membrane, where the yield of polymersomes increased with the diameter of the nanoparticle (Hickey et al., 2014).

1.4.1.1.3 Surface Properties

The surface features of polymersomes are also important as they can determine what kinds of interactions can be associated with biological systems. For instance, the external surface of polymersomes can be altered to show different components (peptides have been utilized in this regard) that enhance their uptake by the cells or help in adhesion to target cells (Leong et al., 2018). One study reported the synthesis of pH-responsive polymersomes that had carboxyl-terminated PEG amphiphile components for enhanced drug penetration inside the cells (Curcio et al., 2018).

1.4.1.2 Analytical Profiling of Polymersome Properties

A range of tools have been identified for the characterization of polymersomes. This type of profiling involves checking various features that are innate to polymersomes, such as the assessment of their physicochemical attributes, including size distribution, morphology, surface charge, encapsulation efficiency, etc. Researchers have employed various techniques for this purpose, such as dynamic light scattering

(DLS), transmission electron microscopy (TEM), zeta potential analysis, Fourier-transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), etc. (Besada et al., 2018; Habel et al., 2015). Cryo-transmission electron microscopy and atomic force microscopy have been found to be particularly useful for smaller diameters.

The systemic analysis provides insight into the structural and functional relationship of polymersomes and is utilized in their optimization for targeted drug delivery and other biomedical applications.

1.4.1.2.1 Electron Microscopy

The use of microscopy can help in the evaluation of important characteristics of polymersomes, including size, morphology, and loading efficiency (J. S. Lee & Feijen, 2012). Because of the small size of polymersomes, electron microscopy is a useful tool in visualization. Scanning electron microscopy (SEM) or transmission electron microscopy (TEM) can visualize the polymersomes with high resolution, but in order to get clear and high-contrast images through these techniques, polymersomes need to be dried (J. S. Lee & Feijen, 2012). Cryogenic TEM (Cryo-TEM) is alternatively used to study polymersomes in a hydrated state for direct imaging (Bermudez et al., 2002; Salva et al., 2013).

1.4.1.2.2 Dynamic Light Scattering

Dynamic Light Scattering (DLS) is a technique used to characterize the size distribution, shapes, and dynamics of macromolecules and colloidal particles, including polymersomes (Abdelmohsen et al., 2016; Stetefeld et al., 2016a). The principle for DLS involves the illumination of the sample by a laser and the detection of the fluctuations of the scattered light caused by the Brownian motion of the particles at a known scattering angle (Stetefeld et al., 2016b). DLS has been previously employed to study polymer motions and molecular weight distribution of

linear and branched polymer systems (Cebe P., 1999; Chu, 1985). DLS also provides the polydispersity index (PDI) for polymersomes, which represents the size distribution, with small PDI values showing good range and stability.

1.4.1.2.3 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is another powerful analytical technique frequently used for structural characterization of polymersomes at the molecular level. NMR spectroscopy has been used in studies to show the membrane dynamics of polymersomes and learn about the structure and composition of polymer chains (Benito et al., 2020). In order to study the interactions of polymersomes with biological membrane and other relevant systems, as well as the encapsulated cargo, NMR-active probes (such as radioisotopes) have been successfully used (Lo & Zeng, 2023).

1.4.1.2.4 Fourier-Transform Infrared Spectroscopy

This technique can give insights into the functional groups available in polymersome components. As FTIR spectroscopy analyzes the absorption bands within the infrared spectrum that correspond to the vibrational modes of respective chemical bonds, it is used to elucidate molecular composition as well as the interaction and compatibility between the encapsulated cargo and nanocarrier components (Alqahtani et al., 2020; Nomani et al., 2017).

1.4.2 Properties of Polymersomes

Polymersomes are of great interest to researchers because of their highly variable properties that can be manipulated for therapeutics and biomedical research. Having a good understanding of these properties and optimizing them is necessary for the development of effective and personalized drug delivery systems.

1.4.2.1 Stability and Biocompatibility

The structure of polymersomes, especially the membranous features, allows them to be more stable than their liposomal counterparts (Poschenrieder et al., 2018). External factors such as temperature and experimental methodologies can influence the stability of polymersomes and sometimes also cause disintegration (Wang et al., 2022). But the stability be enhanced by manipulating their components; for instance, by increasing the molecular weight of the poly (ethylene glycol) polymer, polymersomes showed greatly enhanced stability (Y. Lee et al., 2006). Other studies have shown that by using techniques like size exclusion chromatography and extrusion, stability can be further improved (Bartenstein et al., 2016). Hydrophilic block polymers like PEG are able to impart biocompatibility to polymersomes making them favorable delivery vehicles in therapeutics (G.-Y. Liu et al., 2012). This property also protects polymersomes from being eliminated by natural defense systems like phagocytes, which increase their circulation time and improve the delivery of drugs to target tumor tissues (Onaca et al., 2009).

1.4.2.2 Encapsulation Efficiency

Encapsulation efficiency relates to the ability of polymersomes to encapsulate and maintain components (such as therapeutic or diagnostic agents) within their core (Jain & Kumar, 2010). Encapsulation can be done either during the preparation process or after (Messager et al., 2014). Direct and indirect approaches, including spectroscopic methods, are used to quantify the encapsulation efficiency of the payload of polymersomes, i.e., what percentage of molecules actually end up within polymersomes from the total amount used in the process (Muso-Cachumba et al., 2023). Several factors also affect the encapsulation efficiency such as preparation methods, polymer composition, etc.

1.4.2.3 Controlled Release Kinetics

Drug release from polymersomes is enabled via diffusion through the membrane driven by a concentration gradient. Different factors influence the release rate, such as size, copolymer composition, membrane stability and permeability (J. S. Lee & Feijen, 2012; Siepmann et al., 2004). The membrane properties of polymersomes can be regulated and tuned through molecular design and the addition of specific components (Le Meins et al., 2011). In one study, the release rate of hydrophilic fluorescein isothiocyanate (FITC)-dextran from polymersomes was reported to be affected by the length and the type of hydrophobic block, with the release following first-order kinetics (Zhou & Feijen, 2008). Adjustable release of encapsulated material has also been demonstrated by changing the copolymer composition that makes polymersomes act as artificial cells, targeting specific sites in the body (Meng et al., 2005). Plasmid DNA as encapsulated payload within polymersomes was reported to be released in response to changes in pH from physiological to endocytic conditions (Lomas et al., 2011). Strategies like photo-cross-linking have also been proposed to control membrane phase separation in polymersomes, which can be used to tune the release rate of smaller drugs (S. Chen et al., 2022).

1.4.2.4 Targeting Ligands and Surface Modifications

Polymersomes have been closely studied in the context of drug delivery via nanoreactors, and targeting ligands and surface modifications during the design and preparation step has been a focus to improve their performance (Lefley et al., 2020). Coupling techniques that include Michael addition, biotin-avidin interaction, and click chemistry have shown potential in the biomodification of polymersomes with different ligands (Moulahoum et al., 2021). Cancer cells with their characteristic inflammatory environment, pose a different kind of challenge for the retention of nanocarriers. Functionalized polymersomes with surfaces modified by the conjugation of anti-ICAM-1 antibodies to copolymers showed high binding affinity

to the inflamed cells compared to free polymersomes (J. J. Lin et al., 2006; Robbins et al., 2010). Therefore, the surface modification feature of polymersomes makes them useful carriers for targeted drug delivery.

1.4.2.5 Drug Loading Capacity

Drug loading capacity relates to the ability of polymersomes to accommodate high concentrations of therapeutic agents within their interior. Polymersomes are good candidates for drug delivery because of their stability and ability to load both hydrophilic and hydrophobic molecules (Prakash Jain et al., 2011). Polymersomes have been shown to stably incorporate large hydrophobic molecules, such as pharmaceutical conjugates, in up to 10 mol/wt% concentrations without compromising their stability (Ghoroghchian et al., 2006). Direct self-assembling copolymers have been produced that have a high loading capacity for the model drug aspirin that has poor water solubility and were able to release the drug in a controlled manner (F.-Y. Lin et al., 2018). Nanoprecipitation has also been demonstrated to be a successful method in achieving substantial loading of anticancer drug doxorubicin in poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA) polymersomes (Sanson et al., 2010).

1.4.3 Applications of Polymersomes in Cancer Research

Polymersomes, owing to their unique properties, are of interest for numerous applications in cancer research. The properties of polymersomes can be modified and innovative strategies for combating cancer can be developed, such as overcoming problems of drug resistance, reducing systemic toxicity, and improving treatment outcomes. They hold great promise in personalized medicine and advancing the field of oncology. Some of the recent applications of polymersomes in cancer research are discussed below.

1.4.3.1 Drug Delivery Systems and Therapeutics

The useful features of polymersomes, such as a strong membrane-like structure, ligand conjugation, colloidal stability, and biocompatibility, make them suitable contenders for drug delivery in cancer treatments (Guan et al., 2015). Polymersomes with efficiently encapsulated gold nanorods and doxorubicin (DOX) have been designed to be used as a stimuli-sensitive drug delivery system (DiazDuarte-Rodriguez et al., 2019). Polymersomes can also be designed to have pH-, enzyme-, temperature-, redox-, photo-, and magnetic-field responsive characteristics.

Drug encapsulation within the polymersome is an important factor which is done through both active and passive approaches. In case the water solubility of the drugs is low or high encapsulation efficiency is desired, active loading techniques like salt and pH gradient can be applied (Choucair et al., 2005). Hydrophobic molecules are added to the organic solvent and incorporated into the membrane of the polymersome during the vesicle formation step (Choucair et al., 2005). Hydrophilic drug molecules are added directly to the aqueous phase by dispersing or dissolving with the membrane-forming polymer by a passive loading method (Sharma et al., 2020).

In chemotherapy, administration of drugs like DOX can come with its limitations, such as cardiac myocyte toxicity (Levine et al., 2008). Thus, the use of drug carriers can be an efficient way to overcome systemic toxicities, and polymersomes loaded with drugs in model systems have been researched in this regard. Polymersomes can also act as carriers of DNA, and pH-sensitive DNA-loaded vesicles with a low cytotoxicity and high transfection efficiency are among good candidates for delivery in gene therapy for cancer (Lomas et al., 2007). The biocompatibility and biodegradability of polymersomes favor them in cancer immunotherapy, and they can not only act as immune stimulants but also modify and activate chimeric antigen receptor (CAR)-T cells, deliver immune cargo, and even act as artificial antigen-presenting cells (Le et al., 2022).

1.4.3.2 Polymersomes as Diagnostic Tools

Nanoparticles have been a key interest for scientists for use in diagnostic applications. Polymersomes can hold fluorescent agents that can enable the monitoring of vesicles during drug delivery (Levine et al., 2008). Polymersomes made from polyethylene oxide-*b*-polybutadiene diblock copolymers (PEO-*b*-PBD) with encapsulated porphyrin-based NIRFs (i.e. fluorophores with absorption in the near-infrared spectrum) can generate signals with enough intensity to infiltrate through 1 cm of solid tumor (Ghoroghchian et al., 2005). Such NIR-emissive polymersomes have also been used to label dendritic cells, and when conjugated with a TAT peptide chain, are absorbed in a significant manner by the dendritic cells, allowing for in vivo tracking by fluorescence-based imaging (Christian et al., 2007). Functionalized pH-responsive polymersomes loaded with superparamagnetic iron oxide nanoparticles (SPIONs) and DOX have been formulated, which could be effectively internalized into HeLa cells and exhibited good MRI sensitivity (Chiang et al., 2013). In another study, radiolabeled iodine-rich polymersomes exhibited enhanced circulation time and distribution in breast cancer cells in mice, all the while enabling single-photon emission computed tomography/computed tomography (SPECT/CT) and effectively inhibiting tumor growth (Cao et al., 2019). Iodine-rich polymers were utilized because they are good contrast agents, prevent fast renal excretion, and prolong circulation time (You et al., 2016). So, this shows that the loading of imaging agents into the polymersomes could be helpful for deep-tissue imaging, non-invasive biodistribution and cellular tracking, providing a new robust diagnostic system.

1.4.3.3 Use of Polymersomes in Various Cancers

Numerous studies have been carried out with drug-loaded polymersomes functionalized with specific ligands for targeted cancer therapy. Here, some recent

advances and delivery of therapeutic agents using polymersomes for the treatment of different kinds of cancer will be discussed.

1.4.3.3.1 Breast Cancer

Polymersomes have shown promise in breast cancer treatment. Poly(ethylene glycol)-*block*-poly(ε-caprolactone) (PEG-*b*-PCL) were engineered with peptide to target the p32 protein, which is overexpressed in several tumors including breast cancer, and were able to detect small breast tumors in mice effectively, potentially improving precision-guided tumor imaging and treatment (Simón-Gracia et al., 2018). PEG-PCL polymersomes loaded with Tamoxifen, which is used frequently in breast cancer treatment, and functionalized with iRGD peptides, were helpful in reducing the proliferation of cancer cells, especially tumors with high fibronectin levels (Diaz Bessone et al., 2019). Polyphosphazene polymersomes and Epithelial cell adhesion molecule (EpCAM)-targeted PEG-PLGA polymersomes were shown to enhance the safety and efficacy of DOX delivery in breast cancer treatment (Alibolandi et al., 2015; Xu et al., 2014). Surface-modified poly(dimethylsiloxane)-*b*-poly(methyloxazoline) (PDMS-*b*-PMOXA) polymersomes expressing matrix metalloproteinase 9 (MMP-9), an enzyme involved in cancer progression, loaded with antineoplastic drug paclitaxel showed significantly reduced cell viability and high pharmacological activity in breast cancer cell line (Porta et al., 2018).

1.4.3.3.2 Lung Cancer

For targeted lung cancer therapy, lung cancer-specific chimeric polymersomes have been developed that could deliver pemetrexed, a hydrophilic drug, resulting in effective tumor suppression (Yang, Yang, et al., 2018). Granzyme B-loaded reduction-sensitive polymersomes (GrB-CPRPs) were shown to be effective against lung cancer cells in vivo, inhibiting tumor growth and improving survival rates over the non-targeted and untreated controls (Yang, Wei, et al., 2018). One study reported

that polymersomes encapsulated with cyclic RGD peptide and disulfide-crosslinked DOX (cRGD-PS-DOX) showed good drug loading, high stability, efficient targeting of overexpressed integrin, and inhibited growth of the lung tumors (Zou et al., 2018).

1.4.3.3.3 Brain Cancer

Free exchange of most solutes between plasma and extracellular fluid in the brain is hindered by the blood-brain barrier, and polymersomes expressing different receptors could be potentially used for the efficient transport of drug molecules across the brain endothelium (Sharma et al., 2020). Moreover, the mechanical properties of polymersomes, such as their elasticity, can be tuned to enhance their ability to target brain tumors (Zheng et al., 2021). Their diverse functions also make them effective DDS for treating glioma, a common type of malignant brain tumor (Krishnamoorthy et al., 2014). DOX-loaded polymersomes with Angiopep-2 peptide that can bind to low-density lipoprotein receptor-related protein 1 (LRP1) were devised with high encapsulation efficiency (Figueiredo et al., 2016; F. Lu et al., 2017). These polymersomes had higher toxicity and accumulation in glioma cells in comparison with non-targeted polymersomes (Figueiredo et al., 2016; F. Lu et al., 2017). Docetaxel-loaded polymersomes designed with (PBLG-*b*-HYA) poly (g-benzyl L-glutamate)-block-hyaluronan polymer via nanoprecipitation showed higher in vitro toxicity than free Docetaxel (DTX) in glioblastoma cell lines, which highlights the status of polymersomes for improving DTX therapy (K. K. Upadhyay et al., 2010).

1.4.3.3.4 Liver Cancer

Liver cancer is another lethal malignancy with a very low survival rate. Because of rapid clearance and low tumor uptake of chemotherapeutic agents, treatment is very difficult. Targeted delivery via polymersomes can also be used here. Folate (FA) receptor-targeted polymersomes have been developed that can encapsulate PTX and

DOX, which were shown to be released in a controlled and sustained manner in liver carcinoma cell lines, while suppressing the growth of tumors (Zhu et al., 2017). Moreover, these polymersomes used as combination chemotherapy also exhibited higher tumor growth inhibition than non-targeted ones (Zhu et al., 2017). Chimeric polymersomes loaded with cytochrome C (CC) and functionalized with galactose have exhibited good anti-tumor activity and efficient targeting capacity of proteins into liver cancer cells (Wang et al., 2013).

1.4.3.3.5 Colorectal Cancer

The biocompatibility and controlled biodegradability of polymersomes make them a promising alternative to traditional chemotherapy for colorectal cancer treatment (Maspes et al., 2021). Polymersomes targeted to epidermal growth factor receptor (EGFR) and loaded with plitidepsin have demonstrated high specificity and efficacy in colorectal cancer cell lines (Goñi-de-Cerio et al., 2015). PEG-PLGA polymersomes functionalized with tetraiodothyroacetic acid (Tetrac), that attaches to a specific integrin receptor with high affinity, was shown to release chemotherapeutic drug in a sustained manner with significant toxicity against colorectal cell lines with over-expressing integrins (Alibolandi et al., 2017). Moreover, one study formulated curcumin encapsulated in polymersome nanoparticles (CPNs), and in the evaluation, found a decreased number of stemness markers like CD44, CD133, and CD24, and miRNAs, and increased apoptosis in colorectal cancer cells, making CPNs effective agents for assisted curcumin delivery in colorectal cancer therapy (Pakizehkar et al., 2020).

1.4.3.3.6 Prostate Cancer

Polymersome have also been designed for prostate cancer treatment. Polymersomes assembled from poly(ethylene oxide)-*b*-poly(butadiene) (PEO-*b*-PBD) diblock copolymers and conjugated with a targeting peptide were found to be effectively

internalized within prostate cancer cells and could deliver tumor necrosis factor-α (TNF- α), model protein therapeutic agent (Demirgöz et al., 2009). Folic acid conjugated polymersomes containing anticancer drugs have enhanced tumor-targeting efficiency and internalization into pancreatic cancer cell spheroids as well as reduced cell viability compared with control and free drugs (Karandish et al., 2016).

1.4.3.4 Limitations and Challenges

Polymersomes have a relatively low loading capacity for hydrophilic molecules (Zhao, 2017). In order to be useful delivery vehicles, polymersomes must have high encapsulation efficiencies, but this property remains inconsistent as it is significantly affected by the preparation method, composition, etc. Another challenge is the decreased permeability of polymersomes towards macromolecules and ions that can obstruct their use in therapeutic applications (Sharma et al., 2020). But as they have versatile features, this issue can be solved by programming cargo release using different stimuli.

Polymersomes are designed using synthetic building blocks which can pose a challenge for their biocompatibility and toxicity profile (Mumtaz Virk & Reimhult, 2018). After the dissociation of polymer vesicles in the body, there is the risk of toxicity depending on the nature of the membrane-forming polymer block, hindering the full potential of these materials (J. Du & O'Reilly, 2009). Tumor microenvironment is another major limiting factor that can hinder efficacy of polymersomes. The abnormalities in the tumor microenvironment, such as heterogeneity of blood flow, interstitial fluid pressure, and presence of a stiff extracellular matrix, may prevent such nanocarriers from penetrating and reaching the target (Kansız & Elçin, 2023). The scalability for production on a commercial level is another issue that needs to be addressed for polymersomes to be added in the pharmaceuticals.

1.4.4 Preparation of Polymersomes

Various methods have been developed for polymersome production, each with its own advantages and applications. Common methods include the solvent method, direct dissolution, thin film rehydration technique, double emulsion, and electroformation that can help in making polymersomes with controlled release capabilities (Liao et al., 2012). Men et al. introduced a method using extrusion and sonication with organic solvent as a plasticizing agent, which results in small-sized poly(ethylene glycol)-*block*-polystyrene (PEG-*b*-PS) polymersomes suitable for carrying both hydrophobic and hydrophilic cargo (Men et al., 2016). Marsden et al. demonstrated a detergent-aided method for the formation of peptidic polymersomes from PBLG(36)-E block copolymer, which is particularly useful for encapsulating delicate biomacromolecules (Marsden et al., 2010). The solvent injection technique is also proposed for the rapid formation of polymersomes, offering the flexibility to get a variety of vesicle sizes affected by the concentration of polymer and conditions in which the nanostructure is formulated (Yildiz et al., 2007).

1.4.4.1 Double Emulsion Method

One of the widely used methods is the double emulsion method, which is employed in this study, as it forms monodisperse polymersomes (i.e., all structures will have the same degree of polymerization).

Generally, in the first step, the oil-water single emulsion is formed by the addition of deionized water into a polymer solution prepared in dicholormethane (DCM) solvent. Sonication is employed to obtain small droplets of polymers. The dominant solution in this case is oil, so the outer phase is oil, initiating the formation of reverse micelles. The emulsion is formed, and the addition of a larger aqueous phase with continuous stirring triggers the formation of polymersomes. As the second emulsion step, the blocks that are hydrophilic in nature within the core move towards the outer edge, which makes the polymeric vesicle. The solvent needs to be evaporated to

obtain polymersomes. The surfactants used to balance the isotonicity of the double emulsion system are usually polyvinyl alcohol (PVA) or glucose. In methods like solvent shifting, tetrahydrofolate (THF) or dihydrofolate (DHF) is used, but the double emulsion method employs DCM. This is because THF is highly miscible with water, which hinders drop formation with the required interfacial tension between the organic solvent and inner and outer fluids. DCM is immiscible in water, and this property makes it a suitable solvent. Moreover, the double emulsion methodology is useful in controlling the size and morphology of polymersomes by modifying the droplet sizes of fluids.

The double emulsion method provides high encapsulation efficiencies and uniformity in size. Thiele et al. successfully developed a technique using double emulsions with block copolymers to direct the assembly of polymersomes (Thiele et al., 2010). This was further improved by the use of water-in-oil-in-water double emulsion in templates that direct amphiphilic diblock copolymers into monodisperse polymersomes (Shum et al., 2011). One other innovative approach involves the use of hydrophilic polymers dispersed in polar oils to induce osmose-driven diffusion of water into oil droplets, resulting in the formation of a polymer-rich aqueous droplet within, leading to double emulsions formation (Kotha et al., 2023b). Additionally, the fabrication of Janus polymersomes, made from Janus nanoparticles and characterized by biphasic membranes, have been shown to be achieved through the lateral microphase separation of block copolymers within the vesicle membrane, driven by dewetting (i.e., retraction of fluids) of the double emulsions (S. Li et al., 2020).

1.4.5 Poly(2-alkyl-2-oxazoline)s

Poly(2-alkyl-2-oxazoline)s (PAOXs) are a class of biocompatible polymers that have gained attention for their potential in biomedical applications. They tend to offer higher chemical versatility than poly(ethylene glycol) (PEG) and have comparable advantageous characteristics like stealth behavior and biocompatibility, and they can

also be modified to introduce side-chain functionalities (Mees & Hoogenboom, 2015). They have been explored for use in biomaterials science, for instance, in the formulation of therapeutic polymer-protein conjugates, where they have shown promise as an alternative to PEG (Nemati Mahand et al., 2022). Moreover, in comparison to other hydrophilic water-soluble polymers, PAOXs have demonstrated favorable synthetic aspects and biological and physicochemical properties for biomedical applications.

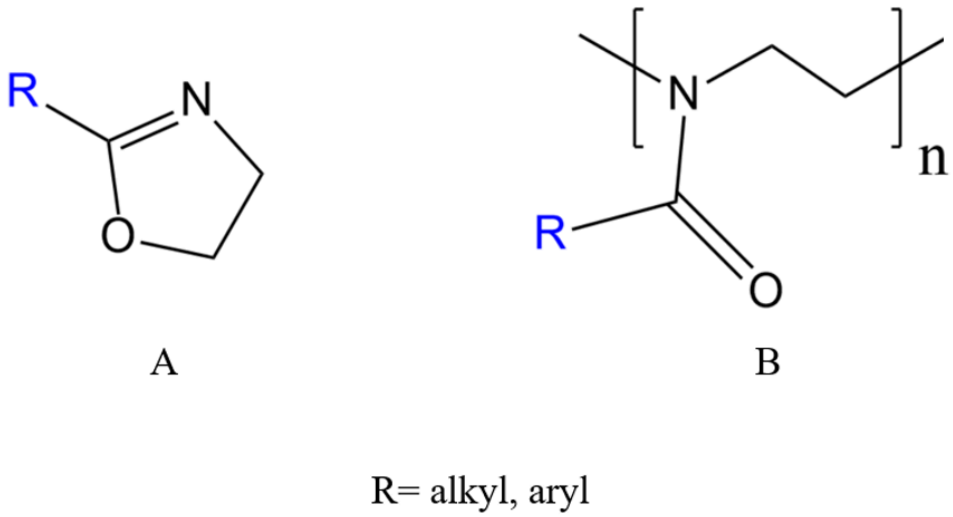


Figure 1.4. Chemical structure of A) 2-alkyl/aryl-2-oxazoline and B) Poly(2-alkyl/aryl-2-oxazoline) PAOXs (Ç. Turan, 2022)

The side chain groups of PAOXs can vary and provide hydrophilic, hydrophobic or thermoresponsive behavior as required. They are usually synthesized via cationic ring opening polymerization (CROP), which allows for the production of well-defined polymers with narrow molar mass distribution and high end-group fidelity (Verbraeken et al., 2014). The main steps in this process are initiation, propagation, and termination. Initiation begins with a nucleophilic attack of nitrogen lone pair on 2-oxazoline onto the initiator, leading to the formation of cationic oxazoline intermediate. Propagation continues afterward with the PAOX backbone when the

monomer attacks the cationic intermediate. The presence of living oxazolinium chains that allows for synthesis of polymers with different and required weight. Nucleophilic terminating agents are used to stop the polymerization process, which attacks the cationic living chain ends (Jana & Hoogenboom, 2022).

The two common types of initiators reported for PAOX synthesis are monofunctional initiators preferred for their higher stability and bifunctional initiators with lower polymerization rates but frequently used for triblock copolymer synthesis (Arraez et al., 2022). The monomer used in CROP is 2-oxazolines, such as 2-phenyl-2-oxazoline (PhOX) and 2-isopropyl-2-oxazoline (iPOX). The chemical nature of monomers is critical for kinetics of these polymerization reactions (Lava et al., 2015). Water, 2-butanol, and aqueous solution of potassium hydroxide are among the common terminating agents used for PAOX polymerization. The use of CROP in the synthesis of PAOXs has been further enhanced by the application of "click chemistry," which enables the introduction of functional groups to the polymers (Lava et al., 2015).

1.4.6 Synthesis and Characterization of PiPOX-*b*-PPhOX-*b*-PiPOX

In this thesis study, the polymersomes were made from triblock copolymer, namely poly(2-isopropyl-2-oxazoline)-*b*-poly(2-phenyl-2-oxazoline)-*b*-poly(2-isopropyl-2-oxazoline) PiPOX-*b*-PPhOX-*b*-PiPOX. The protocols for the synthesis of PiPOX-*b*-PPhOX-*b*-PiPOX and polymersome preparation were developed by Turan et al. (Ç. Turan, 2022; C. Turan et al., 2023).

The synthesis was done by the sequential addition of the monomer through CROP. NMR spectroscopy was used to check and confirm the peaks of the first (PiPOX) and second (PPhOX) blocks. Self-assembly of the triblock copolymer was triggered in aqueous solution through methods like double emulsion and solvent shifting, resulting in aggregates of different sizes. The double emulsion was found to make relatively smaller aggregates with sizes averaging 219.2 nm. The hydrophilic PiPOX

blocks were suggested to form the inner and outer regions of polymersomes, and the hydrophobic PPhOX block formed the membrane of the polymersomes. As the aim was to make a nanoreactor, the encapsulation capability of PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes was also measured using glucose oxidase (GOx) and further analyzed using TEM imaging. The temperature-responsive behavior of the polymersome was also investigated, with a notable irreversible association observed at temperatures above 40°C.

1.5 Glucose Oxidase (GOx) enzyme

Glucose Oxidase (GOx) is a key enzyme that catalyzes the oxidation of glucose to produce gluconic acid and hydrogen peroxide (C. M. Wong et al., 2008). It is a flavoprotein found in various organisms, including fungi, bacteria, and insects, and is commonly used in the food industry, cosmetics, as well as glucose diagnostic kits for detecting glucose in biological solutions (Khatami et al., 2022). GOx is also used in the chemical industry, where it is immobilized on carriers such as carbon nanotubes to enhance its stability and shelf life (Meena et al., 2021). The enzyme's ability to remove oxygen and generate hydrogen peroxide makes it valuable in food preservation and various industrial applications (C. M. Wong et al., 2008).

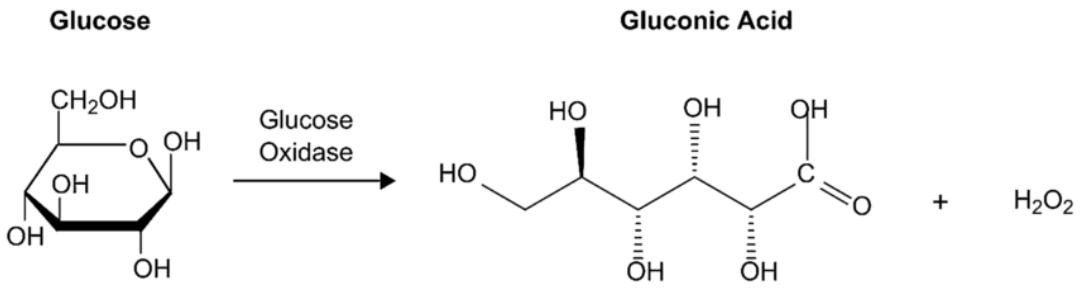


Figure 1.5. The chemical equation of reaction catalyzed by GOx (Sun et al., 2022)

GOx is known for its ability to use oxygen as an external electron acceptor, releasing hydrogen peroxide in the process. Hydrogen peroxide is known for its cytotoxic effects on cancer cells. Polymersomes loaded with GOx can potentially be used to generate hydrogen peroxide within the tumor microenvironment. Incorporating GOx into the polymersomes can also help to exploit its enzymatic activity for targeted drug delivery as an innovative approach. There is limited data on the behavior of PAOX-based block polymersomes in cellular systems, and specifically, their use in combination with GOx for cancer therapy is relatively unexplored.

1.6 Aim of the Thesis

For this study, we hypothesize that PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes loaded with GOx will effectively act as a therapeutic delivery system in colorectal cancer cells. We aimed to examine whether the polymersomes are toxic to human cells and whether they can be used for targeted delivery. We reasoned that the cytotoxic effects of hydrogen peroxide produced by the catalytic reaction of GOx with intracellular glucose may be used as a therapeutic option. In this regard, once the polymersomes were prepared and loaded with GOx, they were tested in a cancer cell line (SW620 colorectal cancer cells) to understand the characteristics and effectiveness of these kinds of nanostructures for targeted drug delivery in cancer treatment and to evaluate the stability and toxicity of the construct itself. In this context, the specific aims of this research are as follows:

- To prepare PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes loaded with GOx
- To characterize and analyze the properties of these polymersomes using different analytical techniques
- To test the in vitro viability studies on a colorectal cancer cell line (SW620)

Self-assembly of different PAOX copolymers into polymersomes has been reported in the literature. However, data on the behavior of such PAOX-based block polymersomes in cellular systems is limited, highlighting the novelty of this research.

CHAPTER 2

EXPERIMENTAL

2.1 Materials

The synthesis and self-assembly of PiPOX-*b*-PPhOX-*b*-PiPOX triblock copolymer have been reported in master's thesis of Cagri Turan and in a recent publication by Turan et al. (Ç. Turan, 2022; C. Turan et al., 2023). Glucose oxidase from Aspergillus niger (EC Number : 232-601-0) and Poly(vinyl alcohol) (Mw 9000-10000) were purchased from Sigma-Aldrich. The deiniozed (DI) water utilized in the experiments was purified by Milli-Q system (Millipore) at 18.2 M Ω.cm.

2.2 Self-assembly of PiPOX-*b*-PPhOX-*b*-PiPOX by double emulsion

The PiPOX-*b*-PPhOX-*b*-PiPOX triblock copolymer was utilized for the preparation of polymersomes via the double emulsion method. 4 mg of PiPOX-*b*-PPhOX-*b*-PiPOX copolymer was dissolved in 0.2 mL of the organic solvent, i.e., DCM. This was followed by dropwise addition of 40 μL of DI water into the solution, with continuous stirring at 200 rpm. Sonication was performed for 3 minutes in order to homogenize the water-in-oil emulsion. Under continuous stirring at 200 rpm, 0.4 mL of 0.1% (w/v) PVA was added in this emulsion. The samples were sonicated for 30 minutes to emulsify the mixture. 2 mL of 0.1% (w/v) PVA solution was further added to dilute the emulsion under stirring. Next, 3 minutes of sonication was carried out to homogenize the emulsion. In the next step, the emulsion was constantly stirred at 25 °C for 2 hours (200 rpm) in an open vial in order to evaporate the DCM. Once the solvent had evaporated, the polymersomes were collected by centrifugation at 6000 rpm for 10 minutes. The supernatant was discarded, followed by the addition of DI water to rinse the particles, followed by centrifugation.

Next, the particles were vortexed for 10 minutes at 2500 rpm, and their hydrodynamic was measured in a Zetasizer Nano-ZS as described below. The polymersomes were filtered using a 5 ml needle syringe containing a 0.45 μm hydrophobic PTFE filter and sterilized under UV light for 15 minutes.

2.3 Loading of GOx in PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes

4 mg of PiPOX-*b*-PPhOX-*b*-PiPOX was dissolved in 0.2 mL of organic solvent, i.e. DCM. This was followed by the preparation of 2.5 mg/mL GOx solution in DI water and dropwise addition of 40 μL of 2.5 mg/mL GOx into the polymer solution, with continuous stirring at 200 rpm. Sonication was performed for 3 minutes in order to homogenize the water-in-oil emulsion. 0.4 mL of 0.1% (w/v) PVA was added in this emulsion under stirring at 200 rpm. The samples were sonicated for 30 minutes to emulsify the mixture. 2 mL of 0.1% (w/v) PVA solution was further added to dilute the water-in-oil emulsion under stirring (200 rpm). Next, 3 minutes of sonication was carried out to homogenize the emulsion. In the next step, the emulsion was constantly stirred at 25 °C for 2 hours (200 rpm) in an open vial in order to evaporate the DCM. Once the solvent had evaporated, the polymersomes were collected by centrifugation at 6000 rpm for 10 minutes. The supernatant was separated, followed by the addition of DI water to rinse the particles, followed by centrifugation.

Next, the particles were vortexed for 10 minutes at 2500 rpm, and their hydrodynamic size was measured in a Zetasizer Nano-ZS as described below. The polymersomes were filtered using a 5 ml needle syringe containing a 0.45 μm hydrophobic PTFE filter and sterilized under UV light for 15 minutes.

2.4 Instrumentation

Zetasizer Nano-ZS equipment (Malvern Instruments Ltd., UK)

Zetasizer Nano-ZS was employed to calculate the size and zeta potential of the GOx-loaded polymersomes. The Dynamic Light Scattering (DLS) technique measures the speed of particles undergoing the Brownian motion, which is defined as a random movement of particles caused by the collision with the molecules of the solvent. This technique helps in measuring particle diameter, which is affected by a number of factors and conditions, including temperature and preparation technique.

DLS was used to define the polymersome size in an aqeous solution, as well as the Polydispersity Insex (PDI) of the vesicles. For size measurement, the sample was put into glass cuvette with refractive index of 1.590 and polystyrene latex was used as reference material. Equilibration time was set as 0 seconds at 25 °C with a measurement angle of 173°. 10 manual measurements were taken, with 11 runs in each measurement lasting 10 seconds. Water was set as the dispersant in the instrument with a refractive index of 1.330. For zeta potential measurement, 3 measurements were taken with 13 runs in each measurement lasting 10 seconds.

Transmission Electron Microscopy (TEM)

TEM images of polymersomes were acquired using a FEI Tecnai G2 Spirit BioTWIN CTEM that operates at an acceleration voltage of 20-120 kV. To obtain images, a drop from the polymersomes solution was added onto the surface of a grid made up of copper and coated with a carbon substrate. This was followed by air-drying the samples and staining for 20 seconds with 0.2% phosphotungstic acid aqueous solution. DI water was used to rinse the grid and samples were dried in air before taking the images.

Fluorescence Microscopy (FM)

GOx was stained with fluorescein isothiocyanate (FITC) before imaging on a fluorescence microscope. For this, 10 mg/ml GOx solution was prepared in carbonate buffer solution (100 mM sodium carbonate, pH 9.0). 25 μL FITC (dissolved in 10 mg/mL DMSO) was added to the enzyme solution and stirred for 2 hours at room temperature. The reaction was then stirred at 10 °C for an additional 18 hours. The product was dialyzed against water for 4 days to remove unreacted FITC. The GOx-loaded polymersomes were photographed using a Zeiss Axio Scope A1 microscope, which employs a Zeiss filter set 49 DAPI filter under a Zeiss AxioCam MRm camera at a magnification of 40X.

FTIR Spectroscopy

Infrared spectra were obtained by scanning the sample with Nicolet iS10 ATR-FTIR Spectrometer. The freeze-dried polymersome sample was placed in the holder and FTIR spectra were recorded in the data spacing region of 0.482 cm^{-1} at room temperature. 64 scans were taken for each interferogram at 4 cm^{-1} resolution. The spectra were also normalized to remove differences in the peak heights of the spectra obtained under different conditions.

2.5 Measurement of GOx Activity and Encapsulation Efficiency

Enzymatic activity for the free GOx and GOx-loaded polymersomes was measured using cascade enzymatic reactions, and the concentration of both free and encapsulated GOx was kept the same at 8 μg/mL. Using PBS buffer (pH 7.4), 8 mg/mL ABTS, 0.072 mg/mL HRP, and 7 mg/mL β-D-glucose solutions were prepared. Next, 560 μL PBS, 80 μL ABTS, 160 μL HRP, and 80 μL of glucose were added into a cuvette, followed by the addition of 80 μL of free enzyme or GOx-loaded polymersome solution. UV-Vis spectroscopy was used to analyze the

enzymatic reactions, and the absorbance was measured at 414 nm, and baseline correction was performed as needed.

To calculate the amount of GOx loaded in the polymersomes, the amount of unloaded GOx was subtracted from the initial value. To measure the amount of unloaded GOx, polymersomes were precipitated by centrifugation, and the Bradford method was used to determine the GOx amount in the supernatant. Protocol published previously was followed for the preparation of Bradford reagent (Zor & Selinger, 1996). 20 mg of Coomassie brilliant blue G-250 (CBB G-250) was dissolved in 10 mL ethanol, followed by the addition of 20 mL of phosphoric acid (85% w/v). Further dilution was done to 200 mL and using a filtered paper, the solution was filtered. Dilution of Bradford reagent was done 2.5-fold with deionized water. 400ul of deionized water and 100ul of supernatant were added to the semi-micro polystyrene disposable cuvette, respectively. 500ul of diluted CBB G-250 dye solution was added to the mixture and incubated at room temperature for 5 minutes. Afterwards, the absorbance spectra were taken using the UV-Visible Spectroscopy method in the range of 400-800 nm. In the next step, serial dilution was performed using 0.1 mg/mL bovine serum albumin stock solution in deionized water under the same conditions, and encapsulation efficiency was calculated. A corresponding calibration curve was prepared by taking the ratio of absorbance value at 595 nm to 450 nm. The following equation was used for the calculation of encapsulation efficiency:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{encapsulated enzyme mass}}{\text{total enzyme mass}} \times 100$$

2.6 Cell Lines and Cell Culture

The human colorectal cancer cell line SW620 was used to evaluate the cytotoxicity of the polymersomes. This cell line was obtained from American Culture Collection (ATCC) and grown in Leibovitz's L-15 medium (Biochrom) which was further supplemented with 2mM L-glutamine in 100% air incubator at 37 °C, as per ATCC

guidelines. The medium of cell culture was supplemented with 10% Fetal Bovine Serum (FBS) and 1% plasmocin (Biochrom). The Leibovitz L-15 medium lacks sodium bicarbonate, which precludes the requirement for a 5% CO_2 incubator. The medium is buffered by the constituent salts, bases, and amino acids. Critically, the medium lacks glucose, which is substituted by galactose to help maintain the physiological pH.

2.7 Cell Viability Assay (MTT)

To determine cell viability after treatment with polymersomes, an MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] assay was performed as per the manufacturer's instructions (Thermo Fisher, USA). 10,000 cells/well were plated in a 96-well plate and the cells were allowed to attach for 24 hours. The following day, the cell culture medium was removed and replaced with 100 ml of bare and GOx-loaded polymersomes at concentrations of 6.25, 12.5, 25, 50, and 100 μg/mL for 24 hours at 37 °C. 0.05 g MTT was dissolved in 1 mL PBS and diluted further in the cell culture medium. At the end of the incubation period, the cell culture medium was aspirated, and 100 μL of MTT-Medium mixture was added to the wells containing cells. This was followed by incubation for 4 hours, and addition of 100 μL 1%SDS- 0.01M HCl to the wells and incubation for another 16 hours at 37 °C. The absorbance was measured at a wavelength of 570 nm using a Multiskan-GO spectrophotometer (Thermo Fisher, USA).

For determination of the effect of different glucose concentrations on the viability of SW620 cells, 10,000 cells/well were cultured in a 96-well plate in the Leibovitz medium with low and high glucose concentrations, i.e., 1.0 g/L and 4.5 g/L, respectively, for 24 hours, followed by the addition of 100 μL of MTT-Medium mixture for 4 hours. After incubation with 100 μL 1%SDS- 0.01M HCl for 16 hours, absorbance was measured at a wavelength of 570 nm. For comparison of cytotoxicity between GOx-loaded and bare polymersomes in the presence of glucose, the SW620 cells were treated with three different concentrations of bare and GOx-loaded polymersomes, i.e., 25, 50, and 100 μg/mL, in Leibovitz L-15 medium supplemented

with 4.5 g/L of glucose. 10,000 cells/well were plated in a 96-well plate, and were allowed to attach for 24 hours. The MTT assay protocol mentioned above was followed, and absorbance was measured at 570 nm.

2.8 Statistical Analysis

All the experiments were repeated at least twice independently. For data analysis, GraphPad Prism 8.0.1 (GraphPad Software Inc) was used. To evaluate significance, Student's t-test was used, and $p<0.05$ was considered statistically significant.

CHAPTER 3

RESULTS AND DISCUSSION

The polymersomes were characterized for size and zeta potential using the DLS technique, for morphology with TEM and Fluorescence imaging, and for analysis of chemical groups in the structure with FTIR spectroscopy.

3.1 Characterization of size and surface charge of PiPOX-*b*-PPhOX-*b*-PiPOX Bare and GOx-loaded Polymersomes

Initially, in the polymersome preparation, the PiPOX-*b*-PPhOX-*b*-PiPOX was dissolved in DCM at 20 mg/mL concentration for droplet formation aided by continuous stirring. The oil-water emulsion was generated by the addition of 40 µl of DI water and 40 µl of 2.5 mg/mL GOx enzyme into the polymer solution for the bare and GOx-loaded polymersome, respectively. Next, sonication was employed to get small polymer droplets that contained hydrophilic PiPOX in the inner core, hydrophobic PPhOX in the membrane and PiPOX in the outer surface. The addition of 2 mL of 0.1% (w/v) PVA triggered the polymersome formation, as it is a hydrophilic polymer with surfactant properties and stabilizes emulsions by reducing the interfacial tensions between the immiscible phases. PVA has been successfully shown to function as a stabilizer in double emulsion systems (Sander et al., 2012). It helps to orient the hydrophobic polymer towards the oil-water interface, where it self-assembles into a bilayer structure. The analysis of the size of bare and GOx-loaded polymersomes was carried out using the DLS technique GOx-loaded polymersomes.

For bare polymersomes, the size was in the range of both small and large aggregates, i.e., around 250 nm and 1000 nm in size, and as per Figure 3.1 A, higher peaks of around 250 nm suggest a higher concentration of smaller aggregates than the others.

The PDI was observed to be 0.271 for the bare polymersomes, suggesting that the size distribution is narrow, whereas the zeta potential centered around -6.95 mV, indicating that the polymersomes have a relatively uniform surface charge. Ions presenting in the buffer solution may adsorb into the polymer surface, in this case, the coronal PiPOX chain, altering the net charge (Wrobel et al., 2015). The zeta potential was stable over time, indicating that the polymersomes are not disintegrating or aggregating.

For the GOx-loaded polymersomes, the prepared emulsion contained both small and large aggregates of polymersomes coexisting in the solution, as shown in Figure 3.2 A. In the intensity size distribution curve, higher peaks of around 1000 nm indicate higher concentrations of larger aggregates than others. The PDI was 0.3, and the z-average was 513.9±53.88, indicating a broader distribution of molecular weights and suggesting more heterogeneity in the sizes of polymersomes.

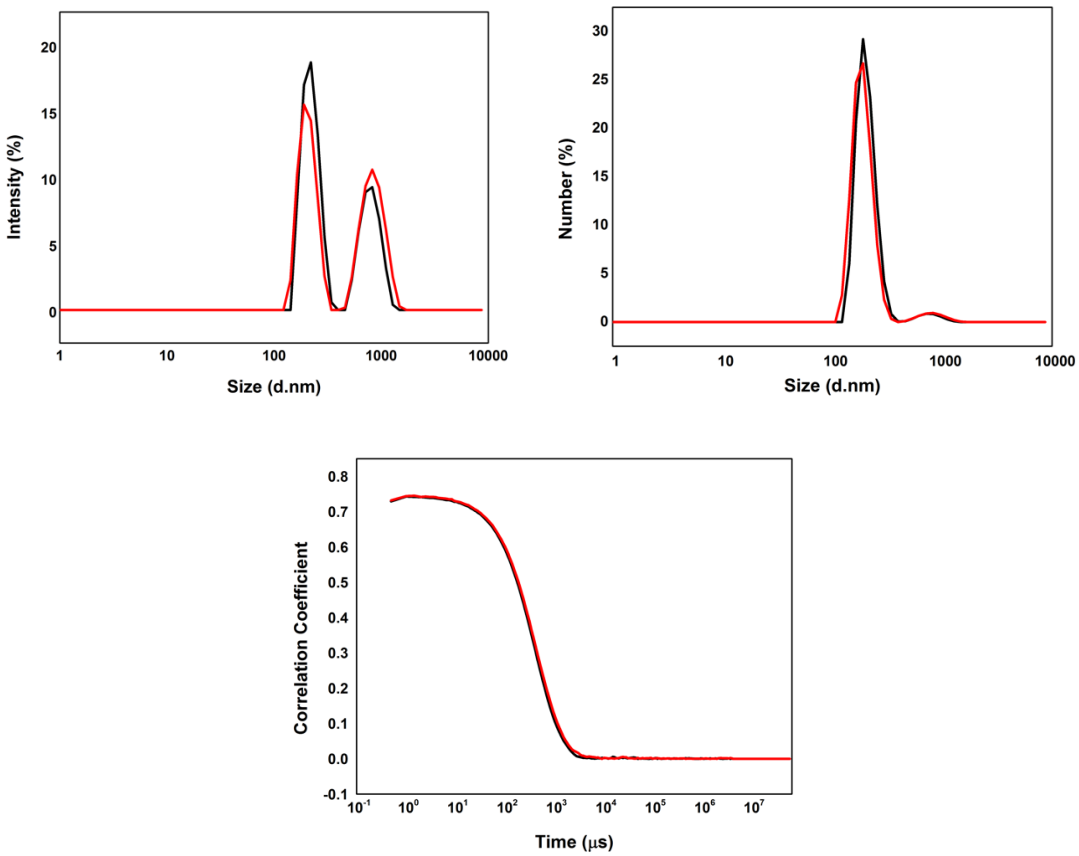
The sizes obtained in the current study varied to a certain extent compared to Turan et al. (Ç. Turan, 2022; C. Turan et al., 2023). The development of PiPOX-*b*-PPhOX-*b*-PiPOX polymersome aggregates with an average size of 219.2 nm using a similar double emulsion method was reported. The difference can be explained by the loss in control of temperature during polymersome preparation. PiPOX shows LCST-type phase behavior at around 39 °C (Konefał et al., 2020). Increasing temperature makes PiPOX outer corona more hydrophobic, which eventually enhances hydrophobic-hydrophobic interactions among the polymersomes and leads to the formation of larger aggregates.

In order to minimize and remove larger aggregates, the solution was also filtered using a 0.4 μm PTFE filter to separate smaller particles, but it should be noted that this step significantly decreases the concentration of polymersomes in a sample. In the number size distribution curve depicted in Figure 3.2 A, the peaks in the range of 100-250 nm indicate reduced aggregation. The sedimentation of polymersomes was also detected during DLS measurements. Sedimentation refers to the settling down of larger particles in the solution, and as they move out of the detection region,

light intensity fluctuates, which affects the size distribution during measurement. This was confirmed during the measurements via the decrease in the mean count rate and non-overlapping time versus correlation coefficient curves.

The light scattering in DLS observes particle motions under an applied electric field to learn about their charge, known as zeta potential. It should be noted that PiPOX is a neutral polymer, and the zeta potential of the polymersomes was taken in PBS buffer with a pH of 7.4. The zeta potential for these polymersomes averaged at -5.31 mV, indicating a slightly negative charge on their surface, which provides a level of electrostatic repulsion between individual polymersomes, helping to reduce aggregation to a certain extent. The negative zeta potential was attributed to the binding of anions in the solution onto the coronal PiPOX chains. There is a possibility that GOx present on the outer surface of polymersomes could contribute towards the negative charge, as it has been reported that pI of GOX was 4.2, so it is expected to be negatively charged at neutral pH (Padilla-Martínez et al., 2015). The zeta potential of GOx in phosphate buffer was reported to be -0.4 mV (Ammam & Fransaer, 2010; Matsumoto et al., 2002).

A



B

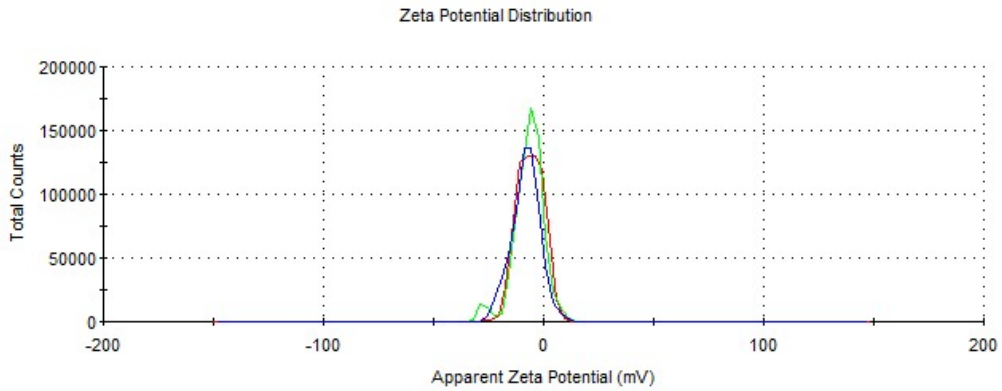


Figure 3.1. A) Size distribution by intensity (top left) and number (top right) of the bare polymersomes, along with the correlation function (bottom middle). B) Zeta Potential distributions of bare polymersomes

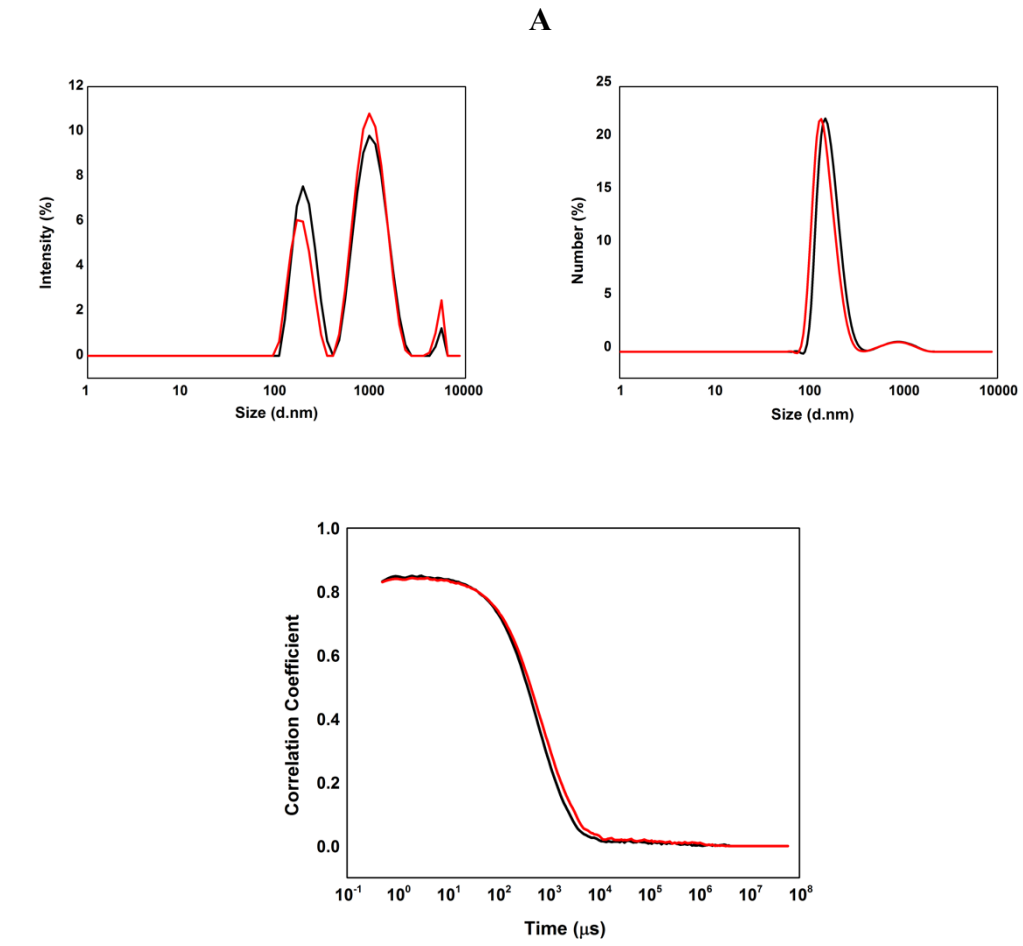


Figure 3.2. A) Size distribution by intensity (top left) and number (top right) of the GOx-loaded polymersomes, along with the correlation function (bottom middle). B) Zeta Potential distributions of GOx-loaded polymersomes

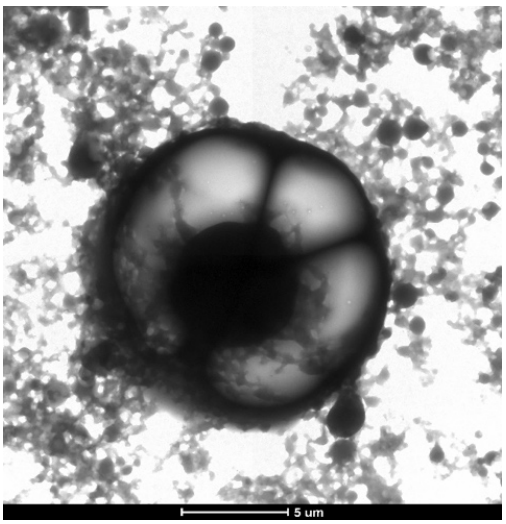
3.2 Morphology of GOx-loaded PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes

The morphology and encapsulation capability of GOx-loaded PiPOX-*b*-PPhOX-*b*-PiPOX polymersome aggregates was also confirmed by TEM imaging and fluorescence microscopy. TEM image of polymersomes showed the presence of stable polymersomes with aggregates in the size range of around 1000 nm and above. In Figure 3.3 A, TEM image of GOx-loaded polymersomes, the dark region can be seen within the particles, which indicates that the enzyme could be located in the hydrophilic core of polymersomes.

Fluorescence microscopy is a dynamic technique and helpful in visualizing big particles. It allows specific labeling and better visualization compared to TEM, in which sample dehydration can affect structural integrity to a certain extent. For fluorescence microscopy, the enzyme was stained with fluorescent isothiocyanate (FITC), and the fluorescence images of the FITC-labelled GOx-loaded polymersomes confirm the association and interaction of GOx with the polymersomes. It is to be noted that, unlike GOx, the triblock copolymer is not fluorescently labeled. Hence, the polymersome shell that encapsulates GOx is not visible under the fluorescence microscope in contrast to the more concentrated fluorescence signal of GOx.

As seen in Figure 3.3 B, fluorescence microscope images clearly show both individual polymersomes as well as aggregates of the polymersomes in a much more defined manner. Moreover, the sizes of polymersomes seen in the confocal images and TEM is in agreement with the DLS data. The DLS data from Figure 3.2 shows the presence of polymersomes with varying sizes, with intensity peaks in 200 nm, 500 nm, and 1000 nm regions. TEM image and fluorescence microscopy demonstrate larger aggregated polymersomes, particularly those around 1000 nm and above. The combined data from these techniques suggest a heterogeneous population of polymersomes, providing a robust validation of the polymersome characterization.

A



B

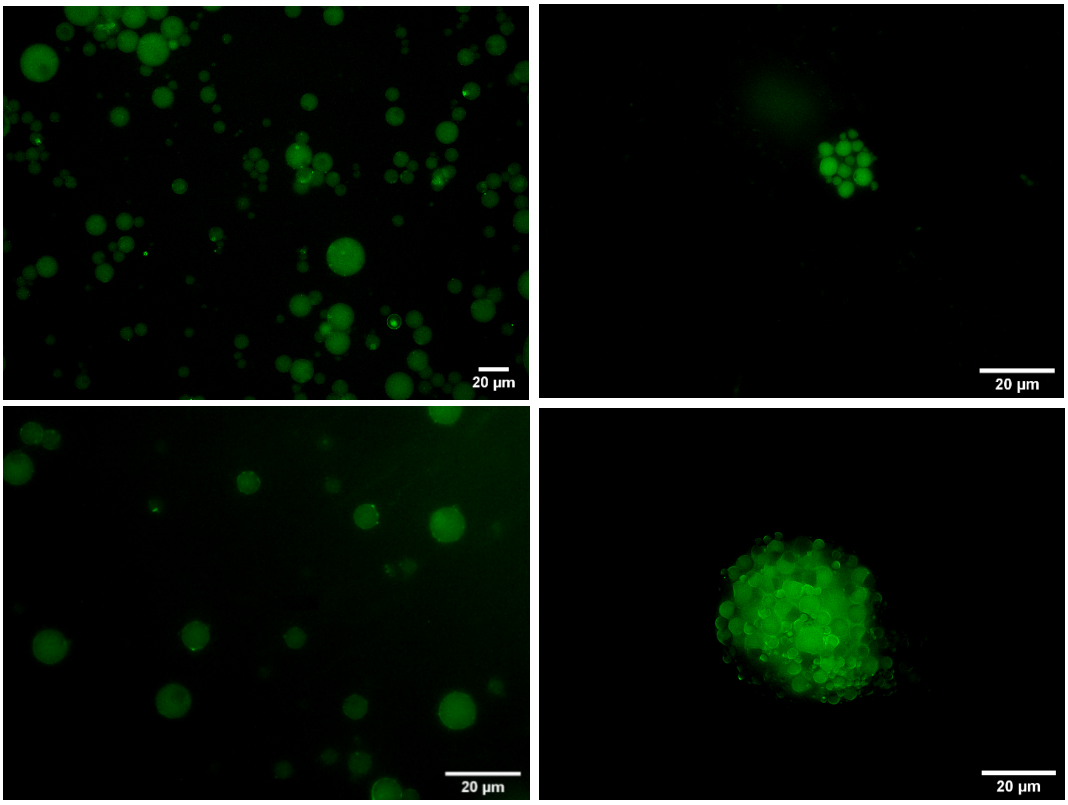


Figure 3.3. A) TEM image of GOx-loaded polymersomes B) Fluorescence microscope images of GOx-loaded polymersomes

3.3 Analysis of the chemical composition of PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes

The FTIR spectrum of the PiPOX-*b*-PPhOX-*b*-PiPOX triblock copolymer was obtained along with the spectra of bare and GOx-loaded polymersomes and compared. In Figure 3.4, the FTIR spectra show that most of the peaks were found between the 900 cm^{-1} to 3500 cm^{-1} region. These peaks indicate stretching for different bonds existing in the compounds. More specifically, we can use the knowledge of peaks across different regions and the associated functional groups and can interpret the data as follows: The peaks at around 1400 cm^{-1} region can be attributed to the C-C and C-H stretching vibrations of PiPOX (Gundogdu et al., 2023). The peaks observed between the region of 2850 cm^{-1} to 3000 cm^{-1} correspond to antisymmetric C-H stretching of -$C(CH_3)_2$, antisymmetric C-H stretching of -CH_2-, and symmetric C-H stretching of -$C(CH_3)_2$ of PiPOX, respectively (Gundogdu et al., 2023), and potentially the CH_2 groups of the PPhOX block (Vlassi et al., 2018).

The spectrum of polymersomes, in comparison with the triblock copolymers, show similarity as they are both made of similar individual components. GOX peaks are reported in the literature. As analyzed by Lv et al., key peaks of unmodified GOX are found at specific wavenumbers corresponding to its functional groups (Lv et al., 2021). These include peaks at around 1630 cm^{-1} and 1540 cm^{-1} corresponding to amide I (C=O) and amide II (N-H and C-H) bands, respectively, characteristic of the protein structures in the peptide backbone of the enzyme. However, characteristic peaks of GOX could not be detected due to the potential contribution of PAOX polymers, which contain similar functional groups. With regards to the bare and GOx-loaded polymersomes, the lack of shifting of existing peaks was correlated with the physical interactions between the functional groups of GOx and the copolymers. Within nanostructures, such interactions of loaded cargo with the particle itself are common, as reported by Mutairi et al., where PEG-grafted liposomes encapsulated with DOX did not show any significant change in stretching band frequencies across

the various functional groups compared to the free liposome, due to the existence of physical rather than chemical interactions (Al Mutairi & Mahmoud Mady, 2022).

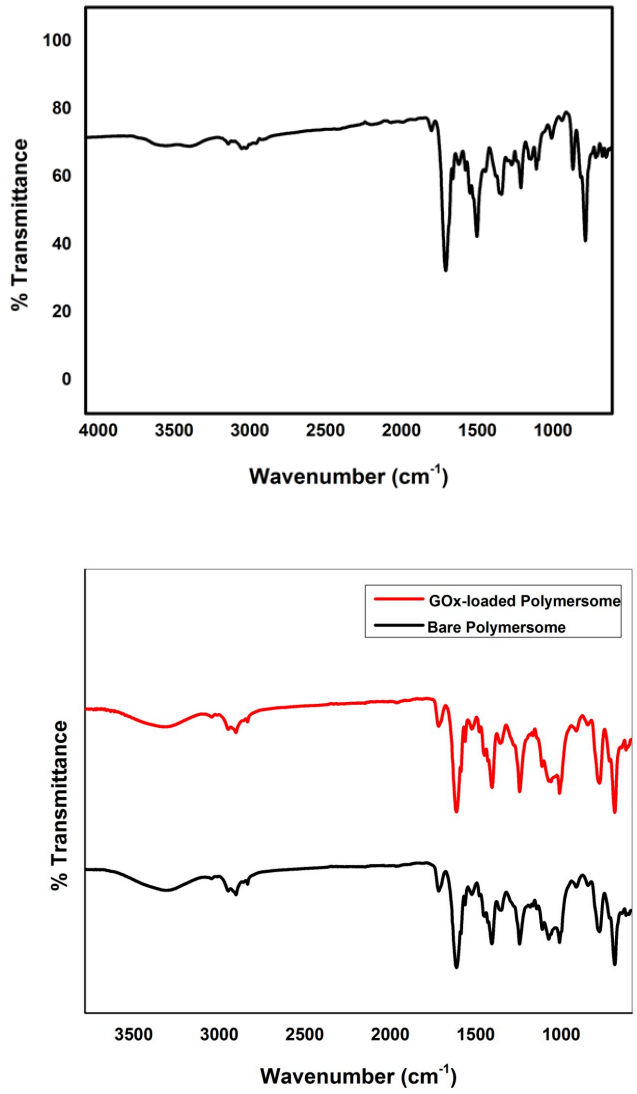


Figure 3.4. FTIR spectra of PiPOX-*b*-PPhOX-*b*-PiPOX triblock copolymer (top) and bare and GOx-loaded polymersomes (bottom)

3.4 Quantification of GOx loading and activity assessment of free and GOx-loaded polymersome

To calculate the amount of GOX loaded in the polymersomes, the amount of unloaded GOX was quantified using the Bradford assay. This was followed by the subtraction of non-encapsulated enzyme from the initial enzyme amount. Figure 3.5 shows the calibration curve prepared with BSA using the Bradford method. The correlation coefficient of the calibration curve was found to be (r^2) = 0.99597. The encapsulation efficiency of GOX was calculated to be 33± 5 %. This moderate encapsulation efficiency indicates that GOx is active. It is to be noted that the encapsulation efficiency may be influenced by several factors, including the method used to prepare the polymersomes and the properties of cargo like its size, charge, etc, that can affect its interaction with the polymersome membrane and its subsequent encapsulation (Parnell et al., 2009).

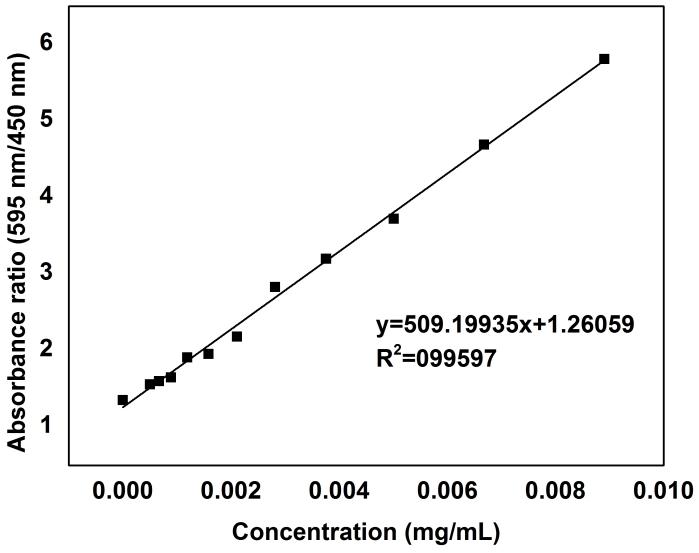


Figure 3.5. Standard curve for GOx depicting encapsulation efficiency using the Bradford assay

The activity of free and polymersome-encapsulated GOx was measured over time to assess the efficiency and kinetics of enzyme release and function. The concentration of both free and encapsulated GOx was kept the same at 8 μg/mL. As shown in Figure 3.6, free GOx exhibited a rapid and linear increase in absorbance, indicating a high level of enzymatic activity. In contrast, GOx-loaded polymersomes show a significantly lower rate. This suggests that while the encapsulated enzyme retains its functional activity, the rate at which it catalyzes the reaction is considerably reduced upon encapsulation within polymersomes compared to the free enzyme.

This reduction in activity is likely attributed to limitations imposed by the polymersome membrane, which hinders substrate accessibility to the enzyme's active site. The encapsulation of the enzyme within the polymersomes likely imposes diffusion limitations or a controlled release mechanism, which could be advantageous for sustained therapeutic applications where a gradual and prolonged enzyme activity is desirable (J. S. Lee & Feijen, 2012).

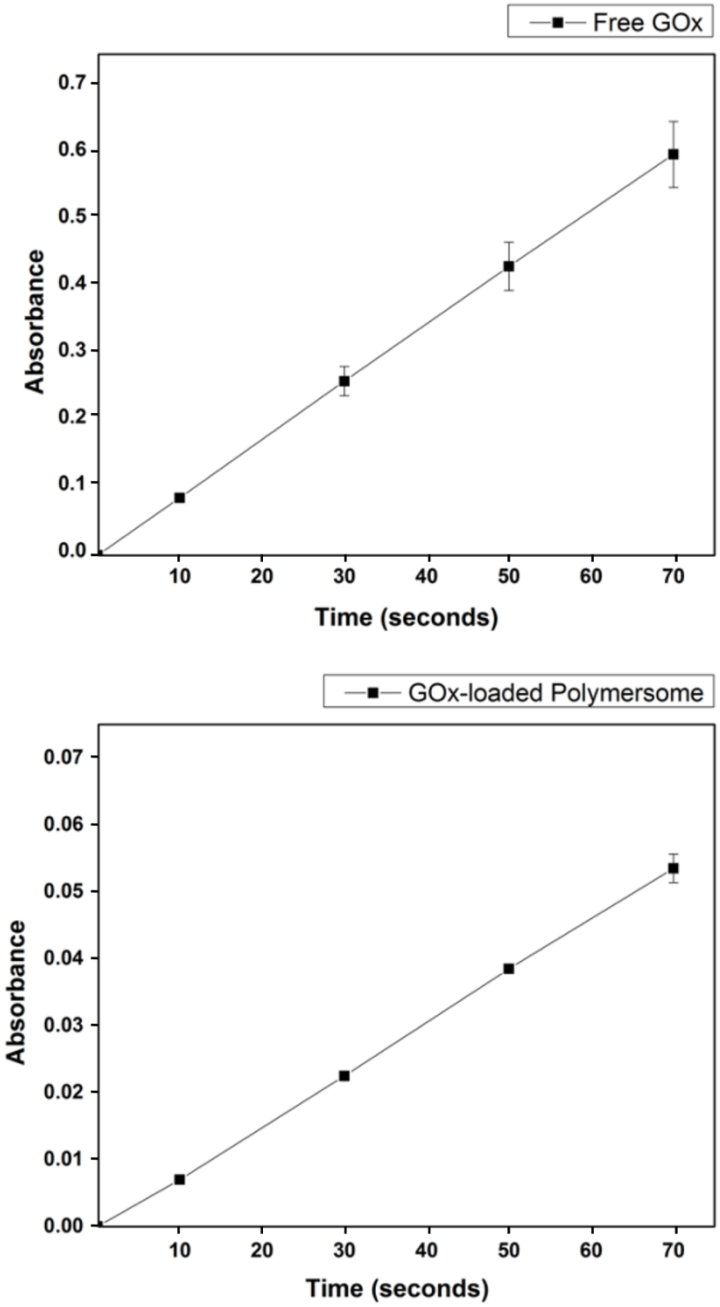


Figure 3.6. Comparison of enzymatic activity between free GOx (top) and GOx-loaded polymersome solution (bottom)

3.5 Evaluation of cytotoxicity of polymersomes in colorectal cancer cell line

As the polymersomes in this study are proposed for potential therapeutic applications, a cell viability assay was carried out to understand and assess the cytotoxicity of polymersomes on the SW620 colorectal cancer cell line. This cell line was selected because its culture medium (Leibovitz L-15) preferentially uses galactose and not glucose as a source of energy. Therefore, any activity of GOx is likely to result from endogenous glucose in the cells, or glucose that is exogenously applied. An MTT assay was carried out to determine cell viability. Five different concentrations, i.e., 6.25, 12.5, 25, 50, and 100 μg/mL for non-filtered bare and GOx-loaded polymersomes, were tested for 24 hours of incubation.

Figure 3.7 shows that the cell viability was above 90% with both bare and GOx-loaded polymersomes. In this study, GOx was specifically used as it can produce the cytotoxic reactive oxygen species hydrogen peroxide, which can be utilized for therapeutic purposes. The consistency of high cell viability across all concentrations, whether low or high, indicates that the tested polymersomes are non-toxic. We concluded that the cytotoxic effects of the polymersomes do not vary significantly with concentration within the tested range. As biocompatibility is a crucial prerequisite for the development of effective therapeutics, the non-toxic nature of the polymersomes indicates that they have the potential to be safe carriers for therapeutic agents. However, it should be noted that from our DLS studies, the polymersomes were found to be in aggregates of various sizes, and because of higher percentage of larger aggregates, cell uptake was not observed. To resolve such an issue, methods of preparation and polymer composition can be adjusted to develop smaller-sized polymersomes that are able to carry cargo inside the cells (Bleul et al., 2015).

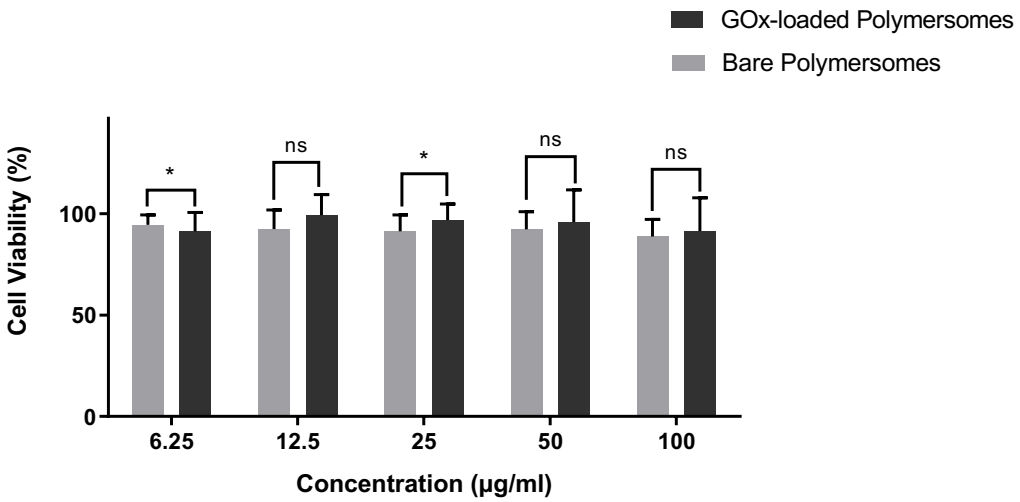


Figure 3.7. Cytotoxic assessments of bare and GOx-loaded PiPOX-*b*-PPhOX-*b*-PiPOX polymersome after 24-hour incubation at 37 °C

*The cells were incubated with five different concentrations of bare and GOx-loaded polymersomes for a 24-hour treatment. The viability of the samples was normalized to CM (complete medium), and percent survival is shown. Statistical analysis was performed using an unpaired t-test (*p<0.05, ns: not significant).*

Next, a preliminary experiment was conducted to investigate the effect of different glucose concentrations on the viability of SW620 cells. The cells were cultured in the Leibovitz L-15 medium supplemented with low and high glucose concentrations, i.e., 1.0 g/L and 4.5 g/L respectively, for 24 hours. As shown in Figure 3.8, cell viability in both groups was around 100%, indicating that the tested glucose concentrations did not have any detrimental effect on the survival of SW620 cells within the 24-hour incubation period. This suggests that SW620 cells can tolerate a wide range of glucose concentrations without experiencing cytotoxicity.

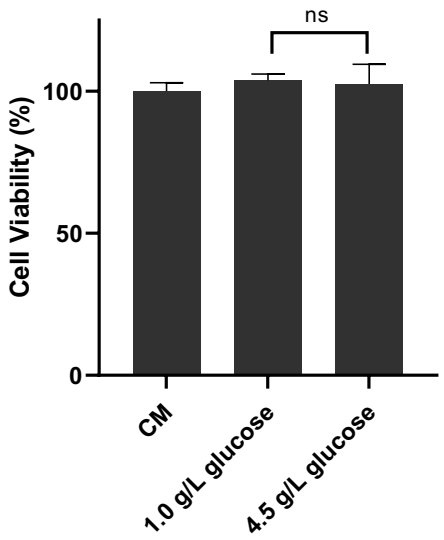


Figure 3.8. Effect of different glucose concentrations on the viability of SW620 cells after 24-hour incubation at 37 °C

The cells were incubated with two different concentrations of glucose for a 24-hour treatment. The viability of the samples was normalized to CM (complete medium), and percent survival is shown Statistical analysis was performed using an unpaired t-test (ns: not significant).

Based on the preliminary findings demonstrating SW620 cell viability across both high and low glucose concentrations, in the following experiment, a higher glucose level of 4.5 g/L was employed to highlight potential differences in cytotoxicity between GOx-loaded and bare polymersomes. The SW620 cells were treated with three different concentrations (25, 50, and 100 µg/mL) of bare and GOx-loaded polymersomes in Leibovitz L-15 medium supplemented with 4.5 g/L of glucose for 24 hours of incubation.

As shown in Figure 3.9, at a concentration of 25 µg/ml, the GOx-loaded polymersomes reduced cell viability by about 20%, while the bare polymersomes had no significant effect. At a concentration of 50 µg/ml, the GOx-loaded polymersomes reduced cell viability by about 50%, while the bare polymersomes only reduced it by about 10%. At a concentration of 100 µg/ml, the GOx-loaded

polymersomes reduced cell viability by about 80%, while the bare polymersomes only reduced it by about 20%. There is a clear increase in cytotoxicity for GOx-loaded polymersomes in the presence of high glucose (4.5 g/L) when compared to both bare polymersomes and the previous experiment without glucose. This suggests that GOx released from the polymersomes outside the cells was active in the high glucose environment, leading to the generation of hydrogen peroxide and therefore enhanced cell death in SW620 cells. As hydrogen peroxide is a reactive oxygen species, it can cause oxidative damage to cellular components, leading to cell death.

These findings highlight the potential of GOx-loaded polymersomes as an effective therapeutic strategy, particularly in high-glucose environments that are characteristic of certain cancerous tissues. The dose-dependent increase in cell death for GOx-loaded polymersomes, compared to the minimal effect of bare polymersomes, emphasizes the critical role of encapsulated GOx and the importance of glucose levels in enhancing the therapeutic efficacy of this delivery system. The interaction between GOx and glucose likely amplifies ROS generation, leading to substantial oxidative stress and subsequent cell death. These results suggest a promising therapeutic potential for GOx-loaded polymersomes in targeted cancer therapies.

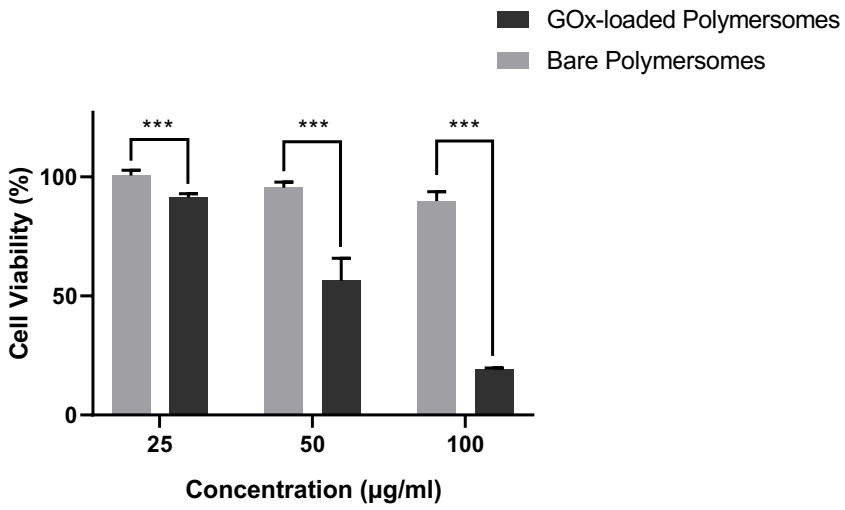


Figure 3.9. Cytotoxic assessments of bare and GOx-loaded PiPOX-*b*-PPhOX-*b*-PiPOX polymersome after 24-hour incubation at 37 °C in Leibovitz L-15 medium supplemented with 4.5 g/L of glucose

Figure 3.9. (cont'd) *The cells were incubated with five different concentrations of bare and GOx-loaded polymersomes for a 24-hour treatment. The viability of the samples was normalized to CM (complete medium), and percent survival is shown. Statistical analysis was performed using an unpaired t-test (***$p<0.001$).*

CHAPTER 4

CONCLUSIONS AND FUTURE PERSPECTIVES

In this study, polymersomes were prepared through self-assembly of PiPOX-*b*-PPhOX-*b*-PiPOX in aqueous solution. GOx was loaded into the polymersomes to exploit its enzymatic activity for producing cytotoxic hydrogen peroxide and also serve as a model enzyme in the development of a safe therapeutic delivery system. Based on the results obtained in this study, GOx could be successfully incorporated into the self-assembled polymersomes and GOx-loaded polymersomes exhibited a toxic nature, supporting the hypothesis.

Double emulsion method was applied for polymersome preparation, and their characterization of morphology was carried out by TEM and fluorescence imaging and for chemical composition by FTIR analysis. Polymersome aggregates were found to have variable sizes ranging from 200 nm to 1000 nm and above. Notably, the intensity percentage for larger aggregates was significant which is why there was no cellular uptake of polymersomes as well. The polymersomes had a core-shell structure in which the hydrophilic PiPOX blocks formed the inner and outer regions, with the hydrophobic PPhOX block forming the membrane. TEM and fluorescence images revealed the successful association of GOx in the inner core region of polymersomes. Cell viability assay was also carried out with these polymersomes using SW620 as the model colorectal cancer cell line. A high cell viability above 90% was consistently observed across all concentrations of bare and GOx-loaded polymersomes, suggesting that the polymersomes are non-toxic within the tested range. A separate experiment explored the potential of these systems in a high-glucose environment (4.5 g/L) relevant to some tumors. In the presence of high glucose, GOx-loaded polymersomes displayed a significant increase in cytotoxicity

compared to bare polymersomes. The likely mechanism involves increased GOx activity due to higher substrate availability, resulting in elevated hydrogen peroxide production and subsequent oxidative stress-induced cell death.

The successful preparation and characterization of PiPOX-*b*-PPhOX-*b*-PiPOX polymersomes for potential application in cancer treatment provide a foundation for future research and development. Some directions for further exploration and optimization based on the results obtained in this study are as follows:

1. The size and surface charge of PiPOX-*b*-PPhOX-*b*-PiPOX GOx-loaded polymersomes revealed heterogeneity in the size distribution with a slightly negative zeta potential. In order to optimize these properties, future studies can focus on fine-tuning the size to minimize the presence of larger aggregates via strategies like adjustment of polymer concentration or functionalization achieved by the addition of favorable groups on the surface. For example, the introduction of specific functional groups to these polymersomes can enhance their targeting capabilities and may also facilitate a controlled release of cargo. Scherer et al. demonstrated that the incorporation of pH-dependent disintegration units and mannose targeting units into the polymersome structure can enhance targeting and cargo release (Scherer et al., 2016). Additionally, surface modification via coating of stimuli-responsive moieties can also help in this regard. So that polymersomes can selectively release their cargo in response to specific tumor microenvironment cues, such as pH, enzymes, or temperature changes.
2. Optimization of the loading process can be carried out to further increase the encapsulation efficiency of GOx within the polymersomes. This can also be further extrapolated for encapsulating other drugs and cargo utilized in theranostic purposes.
3. Further studies are necessary to determine the cellular uptake mechanisms of polymersomes and their intracellular fate. Understanding their interactions at the molecular level will help optimize their design for enhanced therapeutic efficacy.

4. As the evaluation of polymersome cytotoxicity on the SW620 colorectal cancer cell line in the absence of glucose demonstrated high cell viability and the non-toxic nature of polymersomes, targeted studies to assess the biocompatibility and therapeutic efficiency of these drug delivery systems across different models can be performed. It also indicates that these nanocarriers could be used to deliver anticancer drugs or enzymes without causing significant harm to healthy tissues. Successful loading with enzymes like GOx expands their therapeutic potential, as enzymes can be used to trigger drug release or induce cytotoxic effects selectively within cancer cells. Encapsulation of other therapeutic and diagnostic agents within polymersomes can also be performed. These can include chemotherapy drugs, siRNA, imaging contrast agents, or fluorescent dyes to achieve synergistic effects, overcome drug resistance, and monitor treatment response.

Overall, the results from the thesis highlight a role of polymersomes as promising drug delivery vehicles for cancer therapy as per the viability data. By addressing the highlighted areas of improvement and exploring new strategies, the potential of polymersomes as effective carriers for anticancer agents can be further realized. Similar constructs like liposomes, micelles, and polymeric nanoparticles have also shown great potential as drug delivery systems, and together, these systems may contribute towards the development of innovative and targeted therapies for the treatment of various diseases.

REFERENCES

Abdelmohsen, L. K. E. A., Rikken, R. S. M., Christianen, P. C. M., van Hest, J. C. M., & Wilson, D. A. (2016). Shape characterization of polymersome morphologies via light scattering techniques. *Polymer*, *107*, 445–449. https://doi.org/10.1016/j.polymer.2016.06.067

Abetz, V. (2020). Self-Assembly of Block Copolymers. *Polymers*, *12*(4), 794. https://doi.org/10.3390/polym12040794

Adepu, S., & Ramakrishna, S. (2021). Controlled Drug Delivery Systems: Current Status and Future Directions. *Molecules*, *26*(19), 5905. https://doi.org/10.3390/molecules26195905

Al Mutairi, A. A., & Mahmoud Mady, M. (2022). Biophysical Characterization of (DOX-NPtm): FTIR and DSC Studies. *JOURNAL OF ADVANCES IN PHYSICS*, *20*, 41–47. https://doi.org/10.24297/jap.v20i.9194

Alibolandi, M., Ramezani, M., Sadeghi, F., Abnous, K., & Hadizadeh, F. (2015). Epithelial cell adhesion molecule aptamer conjugated PEG–PLGA nanopolymersomes for targeted delivery of doxorubicin to human breast adenocarcinoma cell line in vitro. *International Journal of Pharmaceutics*, *479*(1), 241–251. https://doi.org/10.1016/j.ijpharm.2014.12.035

Alibolandi, M., Rezvani, R., Farzad, S. A., Taghdisi, S. M., Abnous, K., & Ramezani, M. (2017). Tetrac-conjugated polymersomes for integrin-targeted delivery of camptothecin to colon adenocarcinoma in vitro and in vivo. *International Journal of Pharmaceutics*, *532*(1), 581–594. https://doi.org/10.1016/j.ijpharm.2017.09.039

Alqahtani, M. S., Kazi, M., Ahmad, M. Z., Syed, R., Alsenaidy, M. A., & Albraiki, S. A. (2020). Lignin nanoparticles as a promising vaccine adjuvant and delivery system for ovalbumin. *International Journal of Biological Macromolecules*, *163*, 1314–1322. https://doi.org/10.1016/j.ijbiomac.2020.07.026

AlSawaftah, N., Pitt, W. G., & Husseini, G. A. (2021). Dual-Targeting and Stimuli-Triggered Liposomal Drug Delivery in Cancer Treatment. *ACS Pharmacology & Translational Science*, *4*(3), 1028–1049. https://doi.org/10.1021/acsptsci.1c00066

Ammam, M., & Fransaer, J. (2010). A study on electrodeposition of glucose oxidase from low conductivity solutions. *Electrochimica Acta*, *55*(28), 9125–9131. https://doi.org/10.1016/j.electacta.2010.08.050

Andersen, M. E., Krewski, D., & Withey, J. R. (1993). Physiological pharmacokinetics and cancer risk assessment. *Cancer Letters*, *69*(1), 1–14. https://doi.org/10.1016/0304-3835(93)90025-5

Arraez, F. J., Xu, X., Edeleva, M., Van Steenberge, P. H. M., Marien, Y. W., Jerca, V.-V., Hoogenboom, R., & D'hooge, D. R. (2022). Differences and similarities between mono-, bi- or tetrafunctional initiated cationic ring-opening polymerization of 2-oxazolines. *Polymer Chemistry*, *13*(7), 861–876. https://doi.org/10.1039/D1PY01471D

Augustin, R. C., Delgoffe, G. M., & Najjar, Y. G. (2020). Characteristics of the Tumor Microenvironment That Influence Immune Cell Functions: Hypoxia, Oxidative Stress, Metabolic Alterations. *Cancers*, *12*(12), 3802. https://doi.org/10.3390/cancers12123802

Bae, Y. H., & Park, K. (2020). Advanced drug delivery 2020 and beyond: Perspectives on the future. *Advanced Drug Delivery Reviews*, *158*, 4–16. https://doi.org/10.1016/j.addr.2020.06.018

Bartenstein, J. E., Liu, X., Lange, K., Claesson, P. M., & Briscoe, W. H. (2018). Polymersomes at the solid-liquid interface: Dynamic morphological transformation and lubrication. *Journal of Colloid and Interface Science*, *512*, 260–271. https://doi.org/10.1016/j.jcis.2017.10.065

Bartenstein, J. E., Robertson, J., Battaglia, G., & Briscoe, W. H. (2016). Stability of polymersomes prepared by size exclusion chromatography and extrusion.

Colloids and Surfaces A: Physicochemical and Engineering Aspects, *506*, 739–746. https://doi.org/10.1016/j.colsurfa.2016.07.032

Bassyouni, F., ElHalwany, N., Abdel Rehim, M., & Neyfeh, M. (2015). Advances and new technologies applied in controlled drug delivery system. *Research on Chemical Intermediates*, *41*(4), 2165–2200. https://doi.org/10.1007/s11164-013-1338-2

Batty, C. J., Tiet, P., Bachelder, E. M., & Ainslie, K. M. (2019). Drug Delivery for Cancer Immunotherapy and Vaccines. *Pharmaceutical Nanotechnology*, *6*(4), 232–244. https://doi.org/10.2174/2211738506666180918122337

Benito, E., Romero-Azogil, L., Galbis, E., de-Paz, M.-V., & García-Martín, M.-G. (2020). Structurally simple redox polymersomes for doxorubicin delivery. *European Polymer Journal*, *137*, 109952. https://doi.org/10.1016/j.eurpolymj.2020.109952

Benson, H. A., & Prankerd, R. J. (1997). Optimisation of Drug Delivery 2. Principles of Drug Delivery and Limitations of Conventional Non-Oral Dosage Forms. *The Australian Journal of Hospital Pharmacy*, *27*(4), 313–320. https://doi.org/10.1002/jppr1997274313

Bermudez, H., Brannan, A. K., Hammer, D. A., Bates, F. S., & Discher, D. E. (2002). Molecular Weight Dependence of Polymersome Membrane Structure, Elasticity, and Stability. *Macromolecules*, *35*(21), 8203–8208. https://doi.org/10.1021/ma020669l

Besada, L. N., Peruzzo, P., Cortizo, A. M., & Cortizo, M. S. (2018). Preparation, characterization, and in vitro activity evaluation of triblock copolymer-based polymersomes for drugs delivery. *Journal of Nanoparticle Research*, *20*(3), 67. https://doi.org/10.1007/s11051-018-4169-7

Bing, Y., & Shing CHO, C. (2017). The highest realm of precision treatment of cancer – Extending from micro-level precision to holistic personalization.

Australasian Medical Journal, *10*(11). https://doi.org/10.21767/AMJ.2017.3147

Bleul, R., Thiermann, R., & Maskos, M. (2015). Techniques To Control Polymersome Size. *Macromolecules*, *48*(20), 7396–7409. https://doi.org/10.1021/acs.macromol.5b01500

Braithwaite, D., Boffetta, P., Rebbeck, T. R., & Meyskens, F. (2012). Cancer Prevention for Global Health: A Report from the ASPO International Cancer Prevention Interest Group. *Cancer Epidemiology, Biomarkers & Prevention*, *21*(9), 1606–1610. https://doi.org/10.1158/1055-9965.EPI-12-0848

Butcher, N. J., Mortimer, G. M., & Minchin, R. F. (2016). Unravelling the stealth effect. *Nature Nanotechnology*, *11*(4), 310–311. https://doi.org/10.1038/nnano.2016.6

Cao, J., Wei, Y., Zhang, Y., Wang, G., Ji, X., & Zhong, Z. (2019). Iodine-Rich Polymersomes Enable Versatile SPECT/CT Imaging and Potent Radioisotope Therapy for Tumor in Vivo. *ACS Applied Materials & Interfaces*, *11*(21), 18953–18959. https://doi.org/10.1021/acsami.9b04294

Cebe P., H. B. , L. D. (1999). *Scattering from Polymers* (P. Cebe, B. S. Hsiao, & D. J. Lohse, Eds.; Vol. 739). American Chemical Society. https://doi.org/10.1021/bk-2000-0739

Chang, H.-Y., Sheng, Y.-J., & Tsao, H.-K. (2014). Structural and mechanical characteristics of polymersomes. *Soft Matter*, *10*(34), 6373. https://doi.org/10.1039/C4SM01092B

Chauhan, V. P., Stylianopoulos, T., Boucher, Y., & Jain, R. K. (2011). Delivery of Molecular and Nanoscale Medicine to Tumors: Transport Barriers and Strategies. *Annual Review of Chemical and Biomolecular Engineering*, *2*(1), 281–298. https://doi.org/10.1146/annurev-chembioeng-061010-114300

Chen, H., & Zhao, Y. (2018). Applications of Light-Responsive Systems for Cancer Theranostics. *ACS Applied Materials & Interfaces*, *10*(25), 21021–21034. https://doi.org/10.1021/acsami.8b01114

Chen, S., Cornel, E. J., & Du, J.-Z. (2022). Controlling Membrane Phase Separation of Polymersomes for Programmed Drug Release. *Chinese Journal of Polymer Science*, *40*(9), 1006–1015. https://doi.org/10.1007/s10118-022-2683-7

Chen, Y., Jungsuwadee, P., Vore, M., Butterfield, D. A., & St. Clair, D. K. (2007). Collateral Damage in Cancer Chemotherapy: Oxidative Stress in Nontargeted Tissues. *Molecular Interventions*, *7*(3), 147–156. https://doi.org/10.1124/mi.7.3.6

Chiang, W.-H., Huang, W.-C., Chang, C.-W., Shen, M.-Y., Shih, Z.-F., Huang, Y.-F., Lin, S.-C., & Chiu, H.-C. (2013). Functionalized polymersomes with outlayered polyelectrolyte gels for potential tumor-targeted delivery of multimodal therapies and MR imaging. *Journal of Controlled Release*, *168*(3), 280–288. https://doi.org/10.1016/j.jconrel.2013.03.029

Choucair, A., Lim Soo, P., & Eisenberg, A. (2005). Active Loading and Tunable Release of Doxorubicin from Block Copolymer Vesicles. *Langmuir*, *21*(20), 9308–9313. https://doi.org/10.1021/la050710o

Christian, N. A., Milone, M. C., Ranka, S. S., Li, G., Frail, P. R., Davis, K. P., Bates, F. S., Therien, M. J., Ghoroghchian, P. P., June, C. H., & Hammer, D. A. (2007). Tat-Functionalized Near-Infrared Emissive Polymersomes for Dendritic Cell Labeling. *Bioconjugate Chemistry*, *18*(1), 31–40. https://doi.org/10.1021/bc0601267

Chu, B. (1985). Light Scattering Studies of Polymer Solutions and Melts. *Polymer Journal*, *17*(1), 225–238. https://doi.org/10.1295/polymj.17.225

Cirillo, G., Peitzsch, C., Vittorio, O., Curcio, M., Farfalla, A., Voli, F., Dubrovska, A., Iemma, F., Kavallaris, M., & Hampel, S. (2019). When polymers meet

carbon nanostructures: expanding horizons in cancer therapy. *Future Medicinal Chemistry*, *11*(16), 2205–2231. https://doi.org/10.4155/fmc-2018-0540

Craig, G. S. W., & Nealey, P. F. (2007). Self-Assembly of Block Copolymers on Lithographically Defined Nanopatterned Substrates. *Journal of Photopolymer Science and Technology*, *20*(4), 511–517. https://doi.org/10.2494/photopolymer.20.511

Cukierman, E., & Khan, D. R. (2010). The benefits and challenges associated with the use of drug delivery systems in cancer therapy. *Biochemical Pharmacology*, *80*(5), 762–770. https://doi.org/10.1016/j.bcp.2010.04.020

Curcio, M., Mauro, L., Naimo, G. D., Amantea, D., Cirillo, G., Tavano, L., Casaburi, I., Nicoletta, F. P., Alvarez-Lorenzo, C., & Iemma, F. (2018). Facile synthesis of pH-responsive polymersomes based on lipidized PEG for intracellular co-delivery of curcumin and methotrexate. *Colloids and Surfaces B: Biointerfaces*, *167*, 568–576. https://doi.org/10.1016/j.colsurfb.2018.04.057

Dadwal, M. (2014). Polymeric Nanoparticles as Promising Novel Carriers for Drug Delivery: An Overview. *Journal of Advanced Pharmacy Education & Research*, *4*(1).

Debela, D. T., Muzazu, S. G., Heraro, K. D., Ndalama, M. T., Mesele, B. W., Haile, D. C., Kitui, S. K., & Manyazewal, T. (2021). New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Medicine*, *9*, 205031212110343. https://doi.org/10.1177/20503121211034366

Demirgöz, D., Pangburn, T. O., Davis, K. P., Lee, S., Bates, F. S., & Kokkoli, E. (2009). PR_b-targeted delivery of tumor necrosis factor-α by polymersomes for the treatment of prostate cancer. *Soft Matter*, *5*(10), 2011. https://doi.org/10.1039/b814217c

Desai, N. (2012). Challenges in Development of Nanoparticle-Based Therapeutics. *The AAPS Journal*, *14*(2), 282–295. https://doi.org/10.1208/s12248-012-9339-4

Diaz Bessone, M. I., Simón-Gracia, L., Scodeller, P., Ramirez, M. de los A., Lago Huvelle, M. A., Soler-Illia, G. J. A. A., & Simian, M. (2019). iRGD-guided tamoxifen polymersomes inhibit estrogen receptor transcriptional activity and decrease the number of breast cancer cells with self-renewing capacity. *Journal of Nanobiotechnology*, *17*(1), 120. https://doi.org/10.1186/s12951-019-0553-4

DiazDuarte-Rodriguez, M., Cortez-Lemus, N. A., Licea-Claverie, A., Licea-Rodriguez, J., & Méndez, E. R. (2019). Dual Responsive Polymersomes for Gold Nanorod and Doxorubicin Encapsulation: Nanomaterials with Potential Use as Smart Drug Delivery Systems. *Polymers*, *11*(6), 939. https://doi.org/10.3390/polym11060939

Diwanay, S., Gautam, M., & Patwardhan, B. (2004). Cytoprotection and Immunomodulation in Cancer Therapy. *Current Medicinal Chemistry-Anti-Cancer Agents*, *4*(6), 479–490. https://doi.org/10.2174/1568011043352704

Du, J., & O'Reilly, R. K. (2009). Advances and challenges in smart and functional polymer vesicles. *Soft Matter*, *5*(19), 3544. https://doi.org/10.1039/b905635a

Du, Y., Zhang, T., Gieseler, D., Schneider, M., Hafner, D., Sheng, W., Li, W., Lange, F., Wegener, E., Amin, I., & Jordan, R. (2020). Facile Fabrication of Bio- and Dual-Functional Poly(2-oxazoline) Bottle-Brush Brush Surfaces. *Chemistry – A European Journal*, *26*(12), 2749–2753. https://doi.org/10.1002/chem.201905326

Eldar-Boock, A., Polyak, D., Scomparin, A., & Satchi-Fainaro, R. (2013). Nano-sized polymers and liposomes designed to deliver combination therapy for cancer. *Current Opinion in Biotechnology*, *24*(4), 682–689. https://doi.org/10.1016/j.copbio.2013.04.014

Elumalai, K., Srinivasan, S., & Shanmugam, A. (2024). Review of the efficacy of nanoparticle-based drug delivery systems for cancer treatment. *Biomedical Technology*, *5*, 109–122. https://doi.org/10.1016/j.bmt.2023.09.001

Fedele, C., Tothill, R. W., & McArthur, G. A. (2014). Navigating the Challenge of Tumor Heterogeneity in Cancer Therapy. *Cancer Discovery*, *4*(2), 146–148. https://doi.org/10.1158/2159-8290.CD-13-1042

Ferrarelli, L. K. (2015). Overcoming drug resistance in cancer. *Science*, *347*(6217), 38.9-39. https://doi.org/10.1126/science.347.6217.38-i

Figueiredo, P., Balasubramanian, V., Shahbazi, M.-A., Correia, A., Wu, D., Palivan, C. G., Hirvonen, J. T., & Santos, H. A. (2016). Angiopep2-functionalized polymersomes for targeted doxorubicin delivery to glioblastoma cells. *International Journal of Pharmaceutics*, *511*(2), 794–803. https://doi.org/10.1016/j.ijpharm.2016.07.066

George, J. T., & Levine, H. (2021). Implications of Tumor–Immune Coevolution on Cancer Evasion and Optimized Immunotherapy. *Trends in Cancer*, *7*(4), 373–383. https://doi.org/10.1016/j.trecan.2020.12.005

Ghoroghchian, P. P., Frail, P. R., Susumu, K., Blessington, D., Brannan, A. K., Bates, F. S., Chance, B., Hammer, D. A., & Therien, M. J. (2005). Near-infrared-emissive polymersomes: Self-assembled soft matter for *in vivo* optical imaging. *Proceedings of the National Academy of Sciences*, *102*(8), 2922–2927. https://doi.org/10.1073/pnas.0409394102

Ghoroghchian, P. P., Lin, J. J., Brannan, A. K., Frail, P. R., Bates, F. S., Therien, M. J., & Hammer, D. A. (2006). Quantitative membrane loading of polymer vesicles. *Soft Matter*, *2*(11), 973. https://doi.org/10.1039/b604212k

Goñi-de-Cerio, F., Thevenot, J., Oliveira, H., Pérez-Andrés, E., Berra, E., Masa, M., Suárez–merino, B., Lecommandoux, S., & Heredia, P. (2015). Cellular Uptake and Cytotoxic Effect of Epidermal Growth Factor Receptor Targeted and Plitidepsin Loaded Co-Polymeric Polymersomes on Colorectal Cancer

Cell Lines. *Journal of Biomedical Nanotechnology*, *11*(11), 2034–2049. https://doi.org/10.1166/jbn.2015.2148

Guan, L., Rizzello, L., & Battaglia, G. (2015). Polymersomes and their applications in cancer delivery and therapy. *Nanomedicine*, *10*(17), 2757–2780. https://doi.org/10.2217/nnm.15.110

Gundogdu, D., Alemdar, C., Turan, C., Hazal Husnugil, H., Banerjee, S., & Erel-Goktepe, I. (2023). Tuning stimuli-responsive properties of alginate hydrogels through layer-by-layer functionalization for dual-responsive dual drug release. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *676*, 132213. https://doi.org/10.1016/j.colsurfa.2023.132213

Habel, J., Ogbonna, A., Larsen, N., Cherré, S., Kynde, S., Midtgaard, S. R., Kinoshita, K., Krabbe, S., Jensen, G. V., Hansen, J. S., Almdal, K., & Hèlix-Nielsen, C. (2015). Selecting analytical tools for characterization of polymersomes in aqueous solution. *RSC Advances*, *5*(97), 79924–79946. https://doi.org/10.1039/C5RA16403F

Haider, T., Pandey, V., Banjare, N., Gupta, P. N., & Soni, V. (2020). Drug resistance in cancer: mechanisms and tackling strategies. *Pharmacological Reports*, *72*(5), 1125–1151. https://doi.org/10.1007/s43440-020-00138-7

Hajjaji, N., & Bougnoux, P. (2013). Selective sensitization of tumors to chemotherapy by marine-derived lipids: A review. *Cancer Treatment Reviews*, *39*(5), 473–488. https://doi.org/10.1016/j.ctrv.2012.07.001

Hickey, R. J., Koski, J., Meng, X., Riggleman, R. A., Zhang, P., & Park, S.-J. (2014). Size-Controlled Self-Assembly of Superparamagnetic Polymersomes. *ACS Nano*, *8*(1), 495–502. https://doi.org/10.1021/nn405012h

Hirai, T., Leolukman, M., Liu, C. C., Han, E., Kim, Y. J., Ishida, Y., Hayakawa, T., Kakimoto, M., Nealey, P. F., & Gopalan, P. (2009). One-Step Direct-Patterning Template Utilizing Self-Assembly of POSS-Containing Block

Copolymers. *Advanced Materials*, *21*(43), 4334–4338. https://doi.org/10.1002/adma.200900518

Hoeben, A., Joosten, E. A. J., & van den Beuken-van Everdingen, M. H. J. (2021). Personalized Medicine: Recent Progress in Cancer Therapy. *Cancers*, *13*(2), 242. https://doi.org/10.3390/cancers13020242

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug Resistance in Cancer: An Overview. *Cancers*, *6*(3), 1769–1792. https://doi.org/10.3390/cancers6031769

HUANG, Y., COLE, S. P. C., CAI, T., & CAI, Y. (2016). Applications of nanoparticle drug delivery systems for the reversal of multidrug resistance in cancer. *Oncology Letters*, *12*(1), 11–15. https://doi.org/10.3892/ol.2016.4596

Jain, J. P., & Kumar, N. (2010). Development of amphotericin B loaded polymersomes based on (PEG)3-PLA co-polymers: Factors affecting size and in vitro evaluation. *European Journal of Pharmaceutical Sciences*, *40*(5), 456–465. https://doi.org/10.1016/j.ejps.2010.05.005

Jana, S., & Hoogenboom, R. (2022). Poly(2-oxazoline)s: a comprehensive overview of polymer structures and their physical properties—an update. *Polymer International*, *71*(8), 935–949. https://doi.org/10.1002/pi.6426

Jia, R., Teng, L., Gao, L., Su, T., Fu, L., Qiu, Z., & Bi, Y. (2021). Advances in Multiple Stimuli-Responsive Drug-Delivery Systems for Cancer Therapy. *International Journal of Nanomedicine*, *Volume 16*, 1525–1551. https://doi.org/10.2147/IJN.S293427

Jiang, Y., Li, Y., & Zhu, B. (2015). T-cell exhaustion in the tumor microenvironment. *Cell Death & Disease*, *6*(6), e1792–e1792. https://doi.org/10.1038/cddis.2015.162

Jianqing, W., & Ping, Z. (2023). Flawed foundation is the root cause of failure of medicine and precludes cures for chronic diseases. *Global Journal of Cancer Therapy*, *9*(1), 001–019. https://doi.org/10.17352/2581-5407.000050

Jin, Y., Dong, Y., Zhang, J., Sun, J., Liu, Y., & Chen, Y. (2021). The toxicity of cell therapy: Mechanism, manifestations, and challenges. *Journal of Applied Toxicology*, *41*(5), 659–667. https://doi.org/10.1002/jat.4100

Joensuu, H. (2000). Novel cancer therapies: more efficacy, less toxicity and improved organ preservation. *Annals of Medicine*, *32*(1), 31–33. https://doi.org/10.3109/07853890008995907

Kakde Deepak, J. D. S. V. K. R. P. A. T. (2011). Cancer Therapeutics-Opportunities, Challenges and Advances in Drug Delivery. *Journal of Applied Pharmaceutical Science*, *1*(9).

Kansız, S., & Elçin, Y. M. (2023). Advanced liposome and polymersome-based drug delivery systems: Considerations for physicochemical properties, targeting strategies and stimuli-sensitive approaches. *Advances in Colloid and Interface Science*, *317*, 102930. https://doi.org/10.1016/j.cis.2023.102930

Karandish, F., Haldar, M. K., You, S., Brooks, A. E., Brooks, B. D., Guo, B., Choi, Y., & Mallik, S. (2016). Prostate-Specific Membrane Antigen Targeted Polymersomes for Delivering Mocetinostat and Docetaxel to Prostate Cancer Cell Spheroids. *ACS Omega*, *1*(5), 952–962. https://doi.org/10.1021/acsomega.6b00126

Kawahara, N., Akaza, H., Roh, J. K., Nakagawara, A., Masui, T., Horie, S., Sugimura, H., & Inoue, H. (2011). Abstract 1840: What are the implications for cancer research of placing cancer on the global health agenda. *Cancer Research*, *71*(8_Supplement), 1840–1840. https://doi.org/10.1158/1538-7445.AM2011-1840

Kawahara, N., Masui, T., Roh, J. K., Hao, X. S., Hill, D., & Akaza, H. (2010). What Should We Do to Raise Awareness on the Issue of Cancer in the Global

Health Agenda? *Japanese Journal of Clinical Oncology*, *40*(Supplement 1), i82–i85. https://doi.org/10.1093/jjco/hyq132

Kesharwani, R., Jaiswal, P., Patel, D. K., & Yadav, P. K. (2023). Lipid-Based Drug Delivery System (LBDDS): An Emerging Paradigm to Enhance Oral Bioavailability of Poorly Soluble Drugs. *Biomedical Materials & Devices*, *1*(2), 648–663. https://doi.org/10.1007/s44174-022-00041-0

Khare, V., Saxena, A. K., & Gupta, P. N. (2015). Toxicology Considerations in Nanomedicine. In *Nanotechnology Applications for Tissue Engineering* (pp. 239–261). Elsevier. https://doi.org/10.1016/B978-0-323-32889-0.00015-7

Khatami, S. H., Vakili, O., Ahmadi, N., Soltani Fard, E., Mousavi, P., Khalvati, B., Maleksabet, A., Savardashtaki, A., Taheri-Anganeh, M., & Movahedpour, A. (2022). Glucose oxidase: Applications, sources, and recombinant production. *Biotechnology and Applied Biochemistry*, *69*(3), 939–950. https://doi.org/10.1002/bab.2165

Kim, J. A. (2003). Targeted therapies for the treatment of cancer. *The American Journal of Surgery*, *186*(3), 264–268. https://doi.org/10.1016/S0002-9610(03)00212-5

Kiparissides, C., & Kammona, O. (2008). Nanotechnology advances in controlled drug delivery systems. *Physica Status Solidi c*, *5*(12), 3828–3833. https://doi.org/10.1002/pssc.200780129

Koltai, T. (2017). Tumor heterogeneity: An overlooked issue in cancer therapeutics. *Integrative Cancer Science and Therapeutics*, *4*(2). https://doi.org/10.15761/ICST.1000236

Konefał, R., Černoch, P., Konefał, M., & Spěváček, J. (2020). Temperature Behavior of Aqueous Solutions of Poly(2-Oxazoline) Homopolymer and Block Copolymers Investigated by NMR Spectroscopy and Dynamic Light Scattering. *Polymers*, *12*(9), 1879. https://doi.org/10.3390/polym12091879

Kotha, R., Kara, D. D., Roychowdhury, R., Tanvi, K., & Rathnanand, M. (2023a). Polymersomes Based Versatile Nanoplatforms for Controlled Drug Delivery and Imaging. *Advanced Pharmaceutical Bulletin*, *13*(2), 218–232. https://doi.org/10.34172/apb.2023.028

Kotha, R., Kara, D. D., Roychowdhury, R., Tanvi, K., & Rathnanand, M. (2023b). Polymersomes Based Versatile Nanoplatforms for Controlled Drug Delivery and Imaging. *Advanced Pharmaceutical Bulletin*, *13*(2), 218–232. https://doi.org/10.34172/apb.2023.028

Krishnamoorthy, B., Karanam, V., Chellan, V. R., Siram, K., Natarajan, T. selvan, & Gregory, M. (2014). Polymersomes as an effective drug delivery system for glioma – a review. *Journal of Drug Targeting*, *22*(6), 469–477. https://doi.org/10.3109/1061186X.2014.916712

Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces*, *75*(1), 1–18. https://doi.org/10.1016/j.colsurfb.2009.09.001

Kuperkar, K., Patel, D., Atanase, L. I., & Bahadur, P. (2022). Amphiphilic Block Copolymers: Their Structures, and Self-Assembly to Polymeric Micelles and Polymersomes as Drug Delivery Vehicles. *Polymers*, *14*(21), 4702. https://doi.org/10.3390/polym14214702

Lava, K., Verbraeken, B., & Hoogenboom, R. (2015). Poly(2-oxazoline)s and click chemistry: A versatile toolbox toward multi-functional polymers. *European Polymer Journal*, *65*, 98–111. https://doi.org/10.1016/j.eurpolymj.2015.01.014

LaVan, D. A., McGuire, T., & Langer, R. (2003). Small-scale systems for in vivo drug delivery. *Nature Biotechnology*, *21*(10), 1184–1191. https://doi.org/10.1038/nbt876

Le Meins, J. F., Sandre, O., & Lecommandoux, S. (2011). Recent trends in the tuning of polymersomes' membrane properties. *The European Physical*

Journal E 2011 34:2, *34*(2), 1–17. https://doi.org/10.1140/EPJE/I2011-11014-Y

Le, T. M. D., Yoon, A.-R., Thambi, T., & Yun, C.-O. (2022). Polymeric Systems for Cancer Immunotherapy: A Review. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.826876

Lee, J. S., & Feijen, J. (2012). Polymersomes for drug delivery: Design, formation and characterization. *Journal of Controlled Release*, *161*(2), 473–483. https://doi.org/10.1016/j.jconrel.2011.10.005

Lee, Y., Chang, J.-B., Kim, H. K., & Park, T. G. (2006). Stability studies of biodegradable polymersomes prepared by emulsion solvent evaporation method. *Macromolecular Research*, *14*(3), 359–364. https://doi.org/10.1007/BF03219095

Lefley, J., Waldron, C., & Becer, C. R. (2020). Macromolecular design and preparation of polymersomes. *Polymer Chemistry*, *11*(45), 7124–7136. https://doi.org/10.1039/D0PY01247E

Lei, Z., Tian, Q., Teng, Q., Wurpel, J. N. D., Zeng, L., Pan, Y., & Chen, Z. (2023). Understanding and targeting resistance mechanisms in cancer. *MedComm*, *4*(3). https://doi.org/10.1002/mco2.265

Leong, J., Teo, J. Y., Aakalu, V. K., Yang, Y. Y., & Kong, H. (2018). Engineering Polymersomes for Diagnostics and Therapy. *Advanced Healthcare Materials*, *7*(8). https://doi.org/10.1002/adhm.201701276

Levine, D. H., Ghoroghchian, P. P., Freudenberg, J., Zhang, G., Therien, M. J., Greene, M. I., Hammer, D. A., & Murali, R. (2008). Polymersomes: A new multi-functional tool for cancer diagnosis and therapy. *Methods*, *46*(1), 25–32. https://doi.org/10.1016/j.ymeth.2008.05.006

Levitin, H. M., Yuan, J., & Sims, P. A. (2018). Single-Cell Transcriptomic Analysis of Tumor Heterogeneity. *Trends in Cancer*, *4*(4), 264–268. https://doi.org/10.1016/j.trecan.2018.02.003

Li, H., & S. Huck, W. T. (2002). Polymers in nanotechnology. *Current Opinion in Solid State and Materials Science*, *6*(1), 3–8. https://doi.org/10.1016/S1359-0286(02)00008-6

Li, L., Yang, W.-W., & Xu, D.-G. (2019). Stimuli-responsive nanoscale drug delivery systems for cancer therapy. *Journal of Drug Targeting*, *27*(4), 423–433. https://doi.org/10.1080/1061186X.2018.1519029

Li, S., Yu, C., & Zhou, Y. (2020). Computational design of Janus polymersomes with controllable fission from double emulsions. *Physical Chemistry Chemical Physics*, *22*(43), 24934–24942. https://doi.org/10.1039/D0CP04561F

Liao, J., Wang, C., Wang, Y., Luo, F., & Qian, Z. (2012). Recent Advances in Formation, Properties, and Applications of Polymersomes. *Current Pharmaceutical Design*, *18*(23), 3432–3441. https://doi.org/10.2174/138161212801227050

Lim Soo, P., & Eisenberg, A. (2004). Preparation of block copolymer vesicles in solution. *Journal of Polymer Science Part B: Polymer Physics*, *42*(6), 923–938. https://doi.org/10.1002/polb.10739

Lin, F.-Y., Cheng, C.-Y., Chuang, Y.-H., & Tung, S.-H. (2018). Polymersomes with high loading capacity prepared by direct self-assembly of block copolymers in drugs. *Polymer*, *134*, 117–124. https://doi.org/10.1016/j.polymer.2017.11.060

Lin, J. J., Ghoroghchian, P. P., Zhang, Y., & Hammer, D. A. (2006). Adhesion of Antibody-Functionalized Polymersomes. *Langmuir*, *22*(9), 3975–3979. https://doi.org/10.1021/la052445c

Liu, F., Han, L., Huang, X., Sang, M., Liu, B., Li, C., Ma, C., Liu, W., Feng, F., & Qu, W. (2018). Reticuloendothelial System Pre-Block Strategy to Improve Tumor Targeting Efficacy for Hyaluronic Acid Related Drug Delivery System. *Journal of Biomedical Nanotechnology*, *14*(10), 1731–1743. https://doi.org/10.1166/jbn.2018.2603

Liu, G., Yang, L., Chen, G., Xu, F., Yang, F., Yu, H., Li, L., Dong, X., Han, J., Cao, C., Qi, J., Su, J., Xu, X., Li, X., & Li, B. (2021). A Review on Drug Delivery System for Tumor Therapy. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.735446

Liu, G.-Y., Chen, C.-J., & Ji, J. (2012). Biocompatible and biodegradable polymersomes as delivery vehicles in biomedical applications. *Soft Matter*, *8*(34), 8811. https://doi.org/10.1039/c2sm25721a

Liu, J., Li, M., Luo, Z., Dai, L., Guo, X., & Cai, K. (2017). Design of nanocarriers based on complex biological barriers in vivo for tumor therapy. *Nano Today*, *15*, 56–90. https://doi.org/10.1016/j.nantod.2017.06.010

Liu, T., Burger, C., & Chu, B. (2003). Nanofabrication in polymer matrices. *Progress in Polymer Science*, *28*(1), 5–26. https://doi.org/10.1016/S0079-6700(02)00077-1

Lo, C. H., & Zeng, J. (2023). Application of polymersomes in membrane protein study and drug discovery: Progress, strategies, and perspectives. *Bioengineering & Translational Medicine*, *8*(1). https://doi.org/10.1002/btm2.10350

Lomas, H., Canton, I., MacNeil, S., Du, J., Armes, S. P., Ryan, A. J., Lewis, A. L., & Battaglia, G. (2007). Biomimetic pH Sensitive Polymersomes for Efficient DNA Encapsulation and Delivery. *Advanced Materials*, *19*(23), 4238–4243. https://doi.org/10.1002/adma.200700941

Lomas, H., Johnston, A. P. R., Such, G. K., Zhu, Z., Liang, K., Van Koeverden, M. P., Alongkornchotikul, S., & Caruso, F. (2011). Polymersome-Loaded

Capsules for Controlled Release of DNA. *Small*, *7*(14), 2109–2119. https://doi.org/10.1002/smll.201100744

Lu, F., Pang, Z., Zhao, J., Jin, K., Li, H., Pang, Q., Zhang, L., & Pang, Z. (2017). Angiopep-2-conjugated poly(ethylene glycol)-co-poly(ε-caprolactone) polymersomes for dual-targeting drug delivery to glioma in rats. *International Journal of Nanomedicine*, *Volume 12*, 2117–2127. https://doi.org/10.2147/IJN.S123422

Lu, Z.-R., & Qiao, P. (2018). Drug Delivery in Cancer Therapy, Quo Vadis? *Molecular Pharmaceutics*, *15*(9), 3603–3616. https://doi.org/10.1021/acs.molpharmaceut.8b00037

Lv, C., Yang, X., Wang, Z., Ying, M., Han, Q., & Li, S. (2021). Enhanced Performance of Bioelectrodes Made with Amination-Modified Glucose Oxidase Immobilized on Carboxyl-Functionalized Ordered Mesoporous Carbon. *Nanomaterials*, *11*(11), 3086. https://doi.org/10.3390/nano11113086

M. Bimbo, L., Peltonen, L., Hirvonen, J., & A. Santos, H. (2012). Toxicological Profile of Therapeutic Nanodelivery Systems. *Current Drug Metabolism*, *13*(8), 1068–1086. https://doi.org/10.2174/138920012802850047

Marsden, H. R., Quer, C. B., Sanchez, E. Y., Gabrielli, L., Jiskoot, W., & Kros, A. (2010). Detergent-Aided Polymersome Preparation. *Biomacromolecules*, *11*(4), 833–838. https://doi.org/10.1021/bm1001763

Marshall, H. (2001). New drug delivery system developed to prevent immune activation. *Trends in Immunology*, *22*(1), 13. https://doi.org/10.1016/S1471-4906(00)01835-4

Martini, A., & Ciocca, C. (2003). Drug delivery systems for cancer drugs. *Expert Opinion on Therapeutic Patents*, *13*(12), 1801–1807. https://doi.org/10.1517/13543776.13.12.1801

Maspes, A., Pizzetti, F., Rossetti, A., Makvandi, P., Sitia, G., & Rossi, F. (2021). Advances in Bio-Based Polymers for Colorectal Cancer Treatment: Hydrogels and Nanoplatforms. *Gels*, *7*(1), 6. https://doi.org/10.3390/gels7010006

Matchett, K., Lynam-Lennon, N., Watson, R., & Brown, J. (2017). Advances in Precision Medicine: Tailoring Individualized Therapies. *Cancers*, *9*(12), 146. https://doi.org/10.3390/cancers9110146

Matsumoto, N., Chen, X., & Wilson, G. S. (2002). Fundamental Studies of Glucose Oxidase Deposition on a Pt Electrode. *Analytical Chemistry*, *74*(2), 362–367. https://doi.org/10.1021/ac015536x

McGranahan, N., & Swanton, C. (2017). Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell*, *168*(4), 613–628. https://doi.org/10.1016/j.cell.2017.01.018

Meena, J., Gupta, A., Ahuja, R., Singh, M., & Panda, A. K. (2021). Recent advances in nano-engineered approaches used for enzyme immobilization with enhanced activity. *Journal of Molecular Liquids*, *338*, 116602. https://doi.org/10.1016/j.molliq.2021.116602

Mees, M. A., & Hoogenboom, R. (2015). Functional Poly(2-oxazoline)s by Direct Amidation of Methyl Ester Side Chains. *Macromolecules*, *48*(11), 3531–3538. https://doi.org/10.1021/acs.macromol.5b00290

Men, Y., Peng, F., Tu, Y., van Hest, J. C. M., & Wilson, D. A. (2016). Methods for production of uniform small-sized polymersome with rigid membrane. *Polymer Chemistry*, *7*(24), 3977–3982. https://doi.org/10.1039/C6PY00668J

Meng, F., Engbers, G. H. M., & Feijen, J. (2005). Biodegradable polymersomes as a basis for artificial cells: encapsulation, release and targeting. *Journal of Controlled Release*, *101*(1–3), 187–198. https://doi.org/10.1016/j.jconrel.2004.09.026

Messager, L., Gaitzsch, J., Chierico, L., & Battaglia, G. (2014). Novel aspects of encapsulation and delivery using polymersomes. *Current Opinion in Pharmacology*, *18*, 104–111. https://doi.org/10.1016/j.coph.2014.09.017

Michaels, A. S. (1974). Therapeutic Systems for Controlled Administration of Drugs: A New Application of Membrane Science. In *Permeability of Plastic Films and Coatings* (pp. 409–421). Springer US. https://doi.org/10.1007/978-1-4684-2877-3_28

Milewska, S., Niemirowicz-Laskowska, K., Siemiaszko, G., Nowicki, P., Wilczewska, A. Z., & Car, H. (2021). Current Trends and Challenges in Pharmacoeconomic Aspects of Nanocarriers as Drug Delivery Systems for Cancer Treatment. *International Journal of Nanomedicine*, *Volume 16*, 6593–6644. https://doi.org/10.2147/IJN.S323831

Minko, T., Dharap, S., Pakunlu, R., & Wang, Y. (2004). Molecular Targeting of Drug Delivery Systems to Cancer. *Current Drug Targets*, *5*(4), 389–406. https://doi.org/10.2174/1389450043345443

Moulahoum, H., Ghorbanizamani, F., Zihnioglu, F., & Timur, S. (2021). Surface Biomodification of Liposomes and Polymersomes for Efficient Targeted Drug Delivery. *Bioconjugate Chemistry*, *32*(8), 1491–1502. https://doi.org/10.1021/acs.bioconjchem.1c00285

Mumtaz Virk, M., & Reimhult, E. (2018). Phospholipase A $_2$ -Induced Degradation and Release from Lipid-Containing Polymersomes. *Langmuir*, *34*(1), 395–405. https://doi.org/10.1021/acs.langmuir.7b03893

Muso-Cachumba, J. J., Ruiz-Lara, G., Monteiro, G., & Rangel-Yagui, C. de O. (2023). Challenges in estimating the encapsulation efficiency of proteins in polymersomes - Which is the best method? *Brazilian Journal of Pharmaceutical Sciences*, *59*. https://doi.org/10.1590/s2175-97902023e23365

Nagaraju, G. P., & Kamal, M. A. (2020). Challenges in the Discovery of Novel Therapeutic Agents in Cancer. *Current Drug Metabolism*, *20*(12), 931–932. https://doi.org/10.2174/1389200220122001020950 06

Nemati Mahand, S., Aliakbarzadeh, S., Moghaddam, A., Salehi Moghaddam, A., Kruppke, B., Nasrollahzadeh, M., & Khonakdar, H. A. (2022). Polyoxazoline: A review article from polymerization to smart behaviors and biomedical applications. *European Polymer Journal*, *178*, 111484. https://doi.org/10.1016/j.eurpolymj.2022.111484

Nguyen, T. T. N., Luong, M. H., Do, M. T., Kieu, D. M., Li, Q., Nguyen, D. T. T., Tong, Q. C., Ledoux-Rak, I., & Lai, N. D. (2014). *Micro and nanostructuration of polymer materials and applications* (N. Kobayashi, F. Ouchen, & I. Rau, Eds.; p. 91710O). https://doi.org/10.1117/12.2064653

Nomani, A., Nosrati, H., Manjili, H., Khesalpour, L., & Danafar, H. (2017). Preparation and Characterization of Copolymeric Polymersomes for Protein Delivery. *Drug Research*, *67*(08), 458–465. https://doi.org/10.1055/s-0043-106051

Nyström, A. M., & Fadeel, B. (2012). Safety assessment of nanomaterials: Implications for nanomedicine. *Journal of Controlled Release*, *161*(2), 403–408. https://doi.org/10.1016/j.jconrel.2012.01.027

Onaca, O., Enea, R., Hughes, D. W., & Meier, W. (2009). Stimuli-Responsive Polymersomes as Nanocarriers for Drug and Gene Delivery. *Macromolecular Bioscience*, *9*(2), 129–139. https://doi.org/10.1002/mabi.200800248

Padilla-Martínez, S. G., Martínez-Jothar, L., Sampedro, J. G., Tristan, F., & Pérez, E. (2015). Enhanced thermal stability and pH behavior of glucose oxidase on electrostatic interaction with polyethylenimine. *International Journal of Biological Macromolecules*, *75*, 453–459. https://doi.org/10.1016/j.ijbiomac.2015.02.005

Pakizehkar, S., Ranji, N., Sohi, A. N., & Sadeghizadeh, M. (2020). Polymersome-assisted delivery of curcumin: A suitable approach to decrease cancer stemness markers and regulate miRNAs expression in HT29 colorectal cancer cells. *Polymers for Advanced Technologies*, *31*(1), 160–177. https://doi.org/10.1002/pat.4759

Park, K. (2014). Controlled drug delivery systems: past forward and future back. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *190*, 3–8. https://doi.org/10.1016/j.jconrel.2014.03.054

Parnell, A. J., Tzokova, N., Topham, P. D., Adams, D. J., Adams, S., Fernyhough, C. M., Ryan, A. J., & Jones, R. A. L. (2009). The efficiency of encapsulation within surface rehydrated polymersomes. *Faraday Discussions*, *143*, 29–46; discussion 81-93. https://doi.org/10.1039/b902574j

Phelps, M. A., & Sparreboom, A. (2014). A Snapshot of Challenges and Solutions in Cancer Drug Development and Therapy. *Clinical Pharmacology & Therapeutics*, *95*(4), 341–346. https://doi.org/10.1038/clpt.2014.15

Porta, F., Ehrsam, D., Lengerke, C., & Meyer zu Schwabedissen, H. E. (2018). Synthesis and Characterization of PDMS–PMOXA-Based Polymersomes Sensitive to MMP-9 for Application in Breast Cancer. *Molecular Pharmaceutics*, *15*(11), 4884–4897. https://doi.org/10.1021/acs.molpharmaceut.8b00521

Poschenrieder, S. T., Schiebel, S. K., & Castiglione, K. (2018). Stability of polymersomes with focus on their use as nanoreactors. *Engineering in Life Sciences*, *18*(2), 101–113. https://doi.org/10.1002/elsc.201700009

Pradal, J., Maudens, P., Gabay, C., Seemayer, C. A., Jordan, O., & Allémann, E. (2016). Effect of particle size on the biodistribution of nano- and microparticles following intra-articular injection in mice. *International Journal of Pharmaceutics*, *498*(1–2), 119–129. https://doi.org/10.1016/j.ijpharm.2015.12.015

Prakash Jain, J., Yenet Ayen, W., & Kumar, N. (2011). Self Assembling Polymers as Polymersomes for Drug Delivery. *Current Pharmaceutical Design*, *17*(1), 65–79. https://doi.org/10.2174/138161211795049822

Prasanna N., T. C. , S. R. , R. B. , N. G. (2013). Novel Delivery Systems in Cancer Chemotherapy. *Journal of Pharmacy and Pharmaceutical Sciences*, *2*(1).

Qi, Y., Li, B., Wang, Y., & Huang, Y. (2017). Synthesis and sequence-controlled self-assembly of amphiphilic triblock copolymers based on functional poly(ethylene glycol). *Polymer Chemistry*, *8*(45), 6964–6971. https://doi.org/10.1039/C7PY01680H

Ramaswami, R., Harding, V., & Newsom-Davis, T. (2013). Novel cancer therapies: treatments driven by tumour biology. *Postgraduate Medical Journal*, *89*(1057), 652–658. https://doi.org/10.1136/postgradmedj-2012-131533

Rideau, E., Dimova, R., Schwille, P., Wurm, F. R., & Landfester, K. (2018). Liposomes and polymersomes: a comparative review towards cell mimicking. *Chemical Society Reviews*, *47*(23), 8572–8610. https://doi.org/10.1039/C8CS00162F

Riess, G., & Hurtrez, G. (1996). Block Copolymers : Syntheses, Colloidal Properties and Application Possibilities of Micellar Systems. In *Solvents and Self-Organization of Polymers* (pp. 33–51). Springer Netherlands. https://doi.org/10.1007/978-94-009-0333-3_3

Robbins, G. P., Saunders, R. L., Haun, J. B., Rawson, J., Therien, M. J., & Hammer, D. A. (2010). Tunable Leuko-polymersomes That Adhere Specifically to Inflammatory Markers. *Langmuir*, *26*(17), 14089–14096. https://doi.org/10.1021/la1017032

Ruff, P., Al-Sukhun, S., Blanchard, C., & Shulman, L. N. (2016). Access to Cancer Therapeutics in Low- and Middle-Income Countries. *American Society of*

Clinical Oncology Educational Book, *36*, 58–65. https://doi.org/10.1200/EDBK_155975

Sachsenhofer, R., Binder, W. H., Farnik, D., & Zirbs, R. (2007). Polymersome-Embedded Nanoparticles. *Macromolecular Symposia*, *254*(1), 375–377. https://doi.org/10.1002/masy.200750854

Salva, R., Le Meins, J.-F., Sandre, O., Brûlet, A., Schmutz, M., Guenoun, P., & Lecommandoux, S. (2013). Polymersome Shape Transformation at the Nanoscale. *ACS Nano*, *7*(10), 9298–9311. https://doi.org/10.1021/nn4039589

Sanadgol, N., & Wackerlig, J. (2020). Developments of Smart Drug-Delivery Systems Based on Magnetic Molecularly Imprinted Polymers for Targeted Cancer Therapy: A Short Review. *Pharmaceutics*, *12*(9), 831. https://doi.org/10.3390/pharmaceutics12090831

Sander, J. S., Isa, L., Rühs, P. A., Fischer, P., & Studart, A. R. (2012). Stabilization mechanism of double emulsions made by microfluidics. *Soft Matter*, *8*(45), 11471. https://doi.org/10.1039/c2sm26700d

Sanson, C., Schatz, C., Le Meins, J.-F., Soum, A., Thévenot, J., Garanger, E., & Lecommandoux, S. (2010). A simple method to achieve high doxorubicin loading in biodegradable polymersomes. *Journal of Controlled Release*, *147*(3), 428–435. https://doi.org/10.1016/j.jconrel.2010.07.123

Saranath, D., & Khanna, A. (2015). Precision/Personalized medicine in cancer. *Biomedical Research Journal*, *2*(1), 1. https://doi.org/10.4103/2349-3666.240615

Scherer, M., Kappel, C., Mohr, N., Fischer, K., Heller, P., Forst, R., Depoix, F., Bros, M., & Zentel, R. (2016). Functionalization of Active Ester-Based Polymersomes for Enhanced Cell Uptake and Stimuli-Responsive Cargo Release. *Biomacromolecules*, *17*(10), 3305–3317. https://doi.org/10.1021/acs.biomac.6b01049

Schiff, D., Wen, P. Y., & van den Bent, M. J. (2009). Neurological adverse effects caused by cytotoxic and targeted therapies. *Nature Reviews Clinical Oncology*, *6*(10), 596–603. https://doi.org/10.1038/nrclinonc.2009.128

Schuchter, L. M., Luginbuhl, W. E., & Meropol, N. J. (1992). The current status of toxicity protectants in cancer therapy. *Seminars in Oncology*, *19*(6), 742–751.

Seo, B. R., DelNero, P., & Fischbach, C. (2014). In vitro models of tumor vessels and matrix: Engineering approaches to investigate transport limitations and drug delivery in cancer. *Advanced Drug Delivery Reviews*, *69–70*, 205–216. https://doi.org/10.1016/j.addr.2013.11.011

Sharma, A. K., Prasher, P., Aljabali, A. A., Mishra, V., Gandhi, H., Kumar, S., Mutalik, S., Chellappan, D. K., Tambuwala, M. M., Dua, K., & Kapoor, D. N. (2020). Emerging era of "somes": polymersomes as versatile drug delivery carrier for cancer diagnostics and therapy. *Drug Delivery and Translational Research*, *10*(5), 1171–1190. https://doi.org/10.1007/s13346-020-00789-2

Shen, J., & Hogen-Esch, T. (2008). Block Copolymer-like Self-Assembly of Fluorocarbon End-Functionalized Polystyrene and Polybutylmethacrylate. *Journal of the American Chemical Society*, *130*(33), 10866–10867. https://doi.org/10.1021/ja803375a

Shi, R., Wang, Y., & Yao, W. (2021). Block Copolymer Synthesis and Application. *2021 3rd International Academic Exchange Conference on Science and Technology Innovation (IAECST)*, 1228–1236. https://doi.org/10.1109/IAECST54258.2021.9695924

Shiravand, Y., Khodadadi, F., Kashani, S. M. A., Hosseini-Fard, S. R., Hosseini, S., Sadeghirad, H., Ladwa, R., O'Byrne, K., & Kulasinghe, A. (2022). Immune Checkpoint Inhibitors in Cancer Therapy. *Current Oncology*, *29*(5), 3044–3060. https://doi.org/10.3390/curroncol29050247

Shum, H. C., Zhao, Y., Kim, S., & Weitz, D. A. (2011). Multicompartment Polymersomes from Double Emulsions. *Angewandte Chemie International Edition*, *50*(7), 1648–1651. https://doi.org/10.1002/anie.201006023

Shyam Sunder, S., Sharma, U. C., & Pokharel, S. (2023). Adverse effects of tyrosine kinase inhibitors in cancer therapy: pathophysiology, mechanisms and clinical management. *Signal Transduction and Targeted Therapy*, *8*(1), 262. https://doi.org/10.1038/s41392-023-01469-6

Siepmann, J., Faisant, N., Akiki, J., Richard, J., & Benoit, J. P. (2004). Effect of the size of biodegradable microparticles on drug release: experiment and theory. *Journal of Controlled Release*, *96*(1), 123–134. https://doi.org/10.1016/j.jconrel.2004.01.011

Simonazzi, A., Cid, A. G., Villegas, M., Romero, A. I., Palma, S. D., & Bermúdez, J. M. (2018). Nanotechnology applications in drug controlled release. In *Drug Targeting and Stimuli Sensitive Drug Delivery Systems* (pp. 81–116). Elsevier. https://doi.org/10.1016/B978-0-12-813689-8.00003-3

Simón-Gracia, L., Scodeller, P., Fuentes, S. S., Vallejo, V. G., Ríos, X., San Sebastián, E., Sidorenko, V., Di Silvio, D., Suck, M., De Lorenzi, F., Rizzo, L. Y., von Stillfried, S., Kilk, K., Lammers, T., Moya, S. E., & Teesalu, T. (2018). Application of polymersomes engineered to target p32 protein for detection of small breast tumors in mice. *Oncotarget*, *9*(27), 18682–18697. https://doi.org/10.18632/oncotarget.24588

Sleijfer, S., & Verweij, J. (2016). Affordability of drugs used in oncology health care. *Nature Reviews Clinical Oncology*, *13*(6), 331–332. https://doi.org/10.1038/nrclinonc.2016.77

Socka, M., Brzezinski, M., Michalski, A., Kacprzak, A., Makowski, T., & Duda, A. (2018). Self-Assembly of Triblock Copolymers from Cyclic Esters as a Tool for Tuning Their Particle Morphology. *Langmuir*, *34*(12), 3701–3710. https://doi.org/10.1021/acs.langmuir.8b00440

Stetefeld, J., McKenna, S. A., & Patel, T. R. (2016a). Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophysical Reviews*, *8*(4), 409–427. https://doi.org/10.1007/s12551-016-0218-6

Stetefeld, J., McKenna, S. A., & Patel, T. R. (2016b). Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophysical Reviews*, *8*(4), 409–427. https://doi.org/10.1007/s12551-016-0218-6

Sun, Y., Ran, H., & Liu, F. (2022). Polymer-Based Materials and their Applications in Image-Guided Cancer Therapy. *Current Medicinal Chemistry*, *29*(8), 1352–1368. https://doi.org/10.2174/0929867328666210616160717

Szakács, G., Paterson, J. K., Ludwig, J. A., Booth-Genthe, C., & Gottesman, M. M. (2006). Targeting multidrug resistance in cancer. *Nature Reviews. Drug Discovery*, *5*(3), 219–234. https://doi.org/10.1038/nrd1984

Tang, S., Ning, Q., Yang, L., Mo, Z., & Tang, S. (2020). Mechanisms of immune escape in the cancer immune cycle. *International Immunopharmacology*, *86*, 106700. https://doi.org/10.1016/j.intimp.2020.106700

Thenuwara, G., Curtin, J., & Tian, F. (2023). Advances in Diagnostic Tools and Therapeutic Approaches for Gliomas: A Comprehensive Review. *Sensors*, *23*(24), 9842. https://doi.org/10.3390/s23249842

Thiele, J., Abate, A. R., Shum, H. C., Bachtler, S., Förster, S., & Weitz, D. A. (2010). Fabrication of Polymersomes using Double-Emulsion Templates in Glass-Coated Stamped Microfluidic Devices. *Small*, *6*(16), 1723–1727. https://doi.org/10.1002/smll.201000798

Tiwari, G., Tiwari, R., Bannerjee, S., Bhati, L., Pandey, S., Pandey, P., & Sriwastawa, B. (2012). Drug delivery systems: An updated review. *International Journal of Pharmaceutical Investigation*, *2*(1), 2. https://doi.org/10.4103/2230-973X.96920

Trotti, A. (1997). Toxicity antagonists in cancer therapy. *Current Opinion in Oncology*, *9*(6), 569–578. https://doi.org/10.1097/00001622-199711000-00013

Turan, Ç. (2022). *Synthesis and self assembly of poly(2-isopropyl-2-oxazoline)-b-poly(2-phenyl-2-oxazoline)-b-poly(2-isopropyl-2-oxazoline)*.

Turan, C., Terzioglu, I., Gundogdu, D., & Erel-Goktepe, I. (2023). Synthesis of poly(2-isopropyl-2-oxazoline)-b-poly(2-phenyl-2-oxazoline)-b-poly(2-isopropyl-2-oxazoline) and its self-assembly into polymersomes: Temperature-dependent aqueous solution behavior. *Materials Today Communications*, *35*, 106094. https://doi.org/10.1016/j.mtcomm.2023.106094

Upadhyay, K. K., Bhatt, A. N., Castro, E., Mishra, A. K., Chuttani, K., Dwarakanath, B. S., Schatz, C., Le Meins, J., Misra, A., & Lecommandoux, S. (2010). In vitro and In vivo Evaluation of Docetaxel Loaded Biodegradable Polymersomes. *Macromolecular Bioscience*, *10*(5), 503–512. https://doi.org/10.1002/mabi.200900415

Upadhyay, S. K., Rai, R. C., Gehtori, R., Paliwal, A., Gautam, P., & Joshi, P. (2017). Drug Resistance in Cancer. In *Drug Resistance in Bacteria, Fungi, Malaria, and Cancer* (pp. 449–473). Springer International Publishing. https://doi.org/10.1007/978-3-319-48683-3_20

Ventola, C. L. (2017). Cancer Immunotherapy, Part 3: Challenges and Future Trends. *P & T : A Peer-Reviewed Journal for Formulary Management*, *42*(8), 514–521.

Verbraeken, B., Lava, K., & Hoogenboom, R. (2014). Poly(2-oxazoline)s. In *Encyclopedia of Polymer Science and Technology* (pp. 1–51). Wiley. https://doi.org/10.1002/0471440264.pst626

Vikulina, A. S., & Volodkin, D. (2019). Editorial for the Special Issue on Self-Assembly of Polymers. *Micromachines*, *10*(8), 519. https://doi.org/10.3390/mi10080519

Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., Lichtor, T., Decker, W. K., Whelan, R. L., Kumara, H. M. C. S., Signori, E., Honoki, K., Georgakilas, A. G., Amin, A., Helferich, W. G., Boosani, C. S., Guha, G., Ciriolo, M. R., Chen, S., … Kwon, B. S. (2015). Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Seminars in Cancer Biology*, *35 Suppl*, S185–S198. https://doi.org/10.1016/j.semcancer.2015.03.004

Vlassi, E., Papagiannopoulos, A., & Pispas, S. (2018). Hydrolyzed Poly(2-plenyl-2-oxazoline)s in Aqueous Media and Biological Fluids. *Macromolecular Chemistry and Physics*, *219*(13). https://doi.org/10.1002/macp.201800047

Vyas, D., Laput, G., & Vyas, A. (2014). Chemotherapy-enhanced inflammation may lead to the failure of therapy and metastasis. *OncoTargets and Therapy*, 1015. https://doi.org/10.2147/OTT.S60114

Wang, X., Hu, J., & Liu, S. (2022). Overcoming the Dilemma of Permeability and Stability of Polymersomes through Traceless Cross-Linking. *Accounts of Chemical Research*, *55*(23), 3404–3416. https://doi.org/10.1021/acs.accounts.2c00442

Wang, X., Sun, H., Meng, F., Cheng, R., Deng, C., & Zhong, Z. (2013). Galactose-Decorated Reduction-Sensitive Degradable Chimaeric Polymersomes as a Multifunctional Nanocarrier To Efficiently Chaperone Apoptotic Proteins into Hepatoma Cells. *Biomacromolecules*, *14*(8), 2873–2882. https://doi.org/10.1021/bm4007248

Ward, R. A., Fawell, S., Floc'h, N., Flemington, V., McKerrecher, D., & Smith, P. D. (2021). Challenges and Opportunities in Cancer Drug Resistance. *Chemical Reviews*, *121*(6), 3297–3351. https://doi.org/10.1021/acs.chemrev.0c00383

Wong, C. K., Lai, R. Y., & Thordarson, P. (2023). Polymersomes: Fundamentals and Shape Transformation Methods. In *Supramolecular Nanotechnology* (pp. 1027–1054). Wiley. https://doi.org/10.1002/9783527834044.ch38

Wong, C. M., Wong, K. H., & Chen, X. D. (2008). Glucose oxidase: natural occurrence, function, properties and industrial applications. *Applied Microbiology and Biotechnology*, *78*(6), 927–938. https://doi.org/10.1007/s00253-008-1407-4

Wrobel, D., Appelhans, D., Signorelli, M., Wiesner, B., Fessas, D., Scheler, U., Voit, B., & Maly, J. (2015). Interaction study between maltose-modified PPI dendrimers and lipidic model membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1848*(7), 1490–1501. https://doi.org/10.1016/j.bbamem.2015.03.033

Wu Han-Chung, C. D.-K. H. C.-T. (2006). Targeted Therapy for Cancer. *Journal of Cancer Molecules*, *2*(2), 57–66.

Wu, S., Zhu, W., Thompson, P., & Hannun, Y. A. (2018). Evaluating intrinsic and non-intrinsic cancer risk factors. *Nature Communications*, *9*(1), 3490. https://doi.org/10.1038/s41467-018-05467-z

Xu, J., Zhao, Q., Jin, Y., & Qiu, L. (2014). High loading of hydrophilic/hydrophobic doxorubicin into polyphosphazene polymersome for breast cancer therapy. *Nanomedicine: Nanotechnology, Biology and Medicine*, *10*(2), 349–358. https://doi.org/10.1016/j.nano.2013.08.004

Yamanaka, R., Sugawara-Narutaki, A., & Takahashi, R. (2023). *In Situ* Monitoring of Polymerization-Induced Self-Assembly and Gelation During the Synthesis of Triblock Copolymers *via* Time-Resolved Small-Angle X-ray Scattering and Rheology. *Macromolecules*, *56*(11), 4354–4361. https://doi.org/10.1021/acs.macromol.3c00444

Yang, W., Wei, Y., Yang, L., Zhang, J., Zhong, Z., Storm, G., & Meng, F. (2018). Granzyme B-loaded, cell-selective penetrating and reduction-responsive polymersomes effectively inhibit progression of orthotopic human lung tumor in vivo. *Journal of Controlled Release*, *290*, 141–149. https://doi.org/10.1016/j.jconrel.2018.10.013

Yang, W., Yang, L., Xia, Y., Cheng, L., Zhang, J., Meng, F., Yuan, J., & Zhong, Z. (2018). Lung cancer specific and reduction-responsive chimaeric polymersomes for highly efficient loading of pemetrexed and targeted suppression of lung tumor in vivo. *Acta Biomaterialia*, *70*, 177–185. https://doi.org/10.1016/j.actbio.2018.01.015

Yildiz, M. E., Prud'homme, R. K., Robb, I., & Adamson, D. H. (2007). Formation and characterization of polymersomes made by a solvent injection method. *Polymers for Advanced Technologies*, *18*(6), 427–432. https://doi.org/10.1002/pat.858

You, S., Jung, H.-Y., Lee, C., Choe, Y. H., Heo, J. Y., Gang, G.-T., Byun, S.-K., Kim, W. K., Lee, C.-H., Kim, D.-E., Kim, Y. Il, & Kim, Y. (2016). High-performance dendritic contrast agents for X-ray computed tomography imaging using potent tetraiodobenzene derivatives. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *226*, 258–267. https://doi.org/10.1016/j.jconrel.2016.01.036

Zagozdzon, R., Winiarska, M., & Firczuk, M. (2022). Immune Evasion as the Main Challenge for Immunotherapy of Cancer. *Cancers*, *14*(15), 3622. https://doi.org/10.3390/cancers14153622

Zhan, W., Alamer, M., & Xu, X. Y. (2018). Computational modelling of drug delivery to solid tumour: Understanding the interplay between chemotherapeutics and biological system for optimised delivery systems. *Advanced Drug Delivery Reviews*, *132*, 81–103. https://doi.org/10.1016/j.addr.2018.07.013

Zhang, K., Fahs, G. B., Aiba, M., Moore, R. B., & Long, T. E. (2014). Nucleobase-functionalized ABC triblock copolymers: self-assembly of supramolecular architectures. *Chem. Commun.*, *50*(65), 9145–9148. https://doi.org/10.1039/C4CC03363A

Zhao, Y. (2017). Asymmetrical Polymer Vesicles for Drug delivery and Other Applications. *Frontiers in Pharmacology*, *8*. https://doi.org/10.3389/fphar.2017.00374

Zheng, M., Du, Q., Wang, X., Zhou, Y., Li, J., Xia, X., Lu, Y., Yin, J., Zou, Y., Park, J. B., & Shi, B. (2021). Tuning the Elasticity of Polymersomes for Brain Tumor Targeting. *Advanced Science*, *8*(20). https://doi.org/10.1002/advs.202102001

Zhou, W., & Feijen, J. (2008). Biodegradable polymersomes for controlled drug release. *Journal of Controlled Release*, *132*(3), e35–e36. https://doi.org/10.1016/j.jconrel.2008.09.022

Zhu, D., Wu, S., Hu, C., Chen, Z., Wang, H., Fan, F., Qin, Y., Wang, C., Sun, H., Leng, X., Kong, D., & Zhang, L. (2017). Folate-targeted polymersomes loaded with both paclitaxel and doxorubicin for the combination chemotherapy of hepatocellular carcinoma. *Acta Biomaterialia*, *58*, 399–412. https://doi.org/10.1016/j.actbio.2017.06.017

Zor, T., & Selinger, Z. (1996). Linearization of the Bradford Protein Assay Increases Its Sensitivity: Theoretical and Experimental Studies. *Analytical Biochemistry*, *236*(2), 302–308. https://doi.org/10.1006/abio.1996.0171

Zou, Y., Wei, J., Xia, Y., Meng, F., Yuan, J., & Zhong, Z. (2018). Targeted chemotherapy for subcutaneous and orthotopic non-small cell lung tumors with cyclic RGD-functionalized and disulfide-crosslinked polymersomal doxorubicin. *Signal Transduction and Targeted Therapy*, *3*(1), 32. https://doi.org/10.1038/s41392-018-0032-7