Nano-NSAIDs & Cancer: A Novel Approach for Commonplace Drugs in Oncology Practice

A research project presented by

Stavros Naoum

for the Interdisciplinary MSc Course in Nanomedicine
in the
National & Kapodistrian University of Athens

Supervisor: Dr. Maria Gazouli

Committee Members: Dr. Natassa Pippa,
Dr. Hector Katifelis

School of Medicine & School of Pharmacy
National & Kapodistrian
University of Athens
ATHENS

Academic Year 2019 – 2020

By signing this report I confirm that I have read the University policy on Plagiarism, the statement on Academic Integrity and the regulation on the presentation of work for formal assessment on the University web site. I state this piece of work is my own and does not contain any unacknowledged work from other sources.

Signature: **Submission Date:**

Acknowledgements

Special thanks to Dr. Maria Gazouli, Dr. Natassa Pippa and Dr. Hector Katifelis, for their assistance and support during the entirety of this project.

Special thanks to all our lecturers at the National & Kapodistrian University of Athens, for providing us with a platform to express our ideas.

Special thanks to Dr. Mojgan Najafzadeh, for inspiring the main idea of this thesis through her innovative research.

Special thanks to my family, for their endless love and support.

Table of Contents

Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs that are amongst the most commonly prescribed drugs on a global scale. NSAIDs are widely known for their activity against inflammation, as well as for their antipyretic and analgesic properties. NSAIDs have been extensively studied for their chemotherapeutic and chemopreventive activity against various types of cancer, underlining their capacity as a commonplace, off-the-shelf anti-tumor agent. The last years have seen a great increase in the use of nanotechnology in modern medicine, especially due to the unique properties of nanoparticles. These properties include their much smaller size, greater surface area and many other unparalleled chemical, optical, and electrical properties. The full anticancer potential of nonsteroidal anti-inflammatory drugs on the nano scale has not been exploited and, for the most part, remains unrealized. This review aims to shed a light on the use of nano-NSAIDs in cancer treatment, suggest future courses of action, and hopefully inspire future research in order to expand current knowledge on the unexplored potential of nano-NSAIDs in chemotherapy.

1.0 Introduction

1.1 Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal Anti-Inflammatory drugs (NSAIDs) are a class of drugs that are primarily used for their anti-inflammatory, antipyretic and analgesic properties. NSAIDs are mainly prescribed for the treatment of common pain and tissue inflammation and are considered to be among the most frequently prescribed medications on a global scale (Jin, 2015). Prescription of nonsteroidal anti-inflammatory drugs accounts for approximately 5% of all medication prescriptions worldwide (Smalley *et al*., 1995; Mizushima, 2010). Many nonsteroidal anti-inflammatory drugs, such as aspirin, are available even in the absence of a prescription (Jin, 2015).

Nonsteroidal anti-inflammatory drugs can be divided into six categories, based on their chemical structure. These six categories of NSAIDs include salicylates, enolic acid derivatives, acetic acid derivatives, propionic acid derivatives, anthranillic acid derivatives, and selective inhibitors of COX-2 (GlobalRPH, n.d.). The first five categories are often referred to as 'non-specific NSAIDs' and are known to inhibit both COX-1 and COX-2 enzyme isoforms, whereas selective inhibitors only target COX-2 (Jin, 2015). Aspirin is the most commonly known example of salicylates, and the most commonly known nonsteroidal anti-inflammatory drug worldwide. Other widely known examples of NSAIDs include meloxicam for the enolic acid derivatives, flufenamic acid for the anthranillic acid derivatives, diclofenac and sulindac for the acetic acid-derived NSAIDs, ibuprofen, naproxen and ketoprofen for the propionic acid derivatives, and celecoxib for the selective COX-s inhibitor NSAIDs (GlobalRPH, n. d.; Jin, 2015).

Intake of low doses of nonsteroidal anti-inflammatory drugs for a brief time period is considered to be safe and generally not cause any significant side effects. Prolonged administration of NSAIDs, however, is often associated with adverse side effects, including gastrointestinal ulceration and bleeding, heart attack, stroke, congestive heart failure, liver failure and kidney disease, especially in patients with a known history of these conditions. COX-2 selective inhibitors are known to cause less adverse side effects to the gastrointestinal tract, compared to their non-selective counterpart. On the other hand, the presence of significant

cardiovascular side effects appears to be more common with the use of COX-2 selective NSAIDs (Whittle, 2003; Jin, 2015; Wongrakpanich *et al*., 2018).

1.2 NSAIDs: Mechanism of Action

The anti-inflammatory potential of nonsteroidal anti-inflammatory drugs is achieved through inhibition of the activity of an enzyme family known as cyclooxygenases (COX) or prostaglandin-endoperoxide synthases (PTGS) (Vane and Botting, 1998; Mizushima, 2010). Cyclooxygenases are an enzyme family comprising membrane-bound haemo- and glycoproteins with a defined molecular weight of 71kD. Scientists have reported that COX enzymes can be found within the endoplasmic reticulum of all prostanoid-forming cell types, including vascular smooth muscle and endothelial cells (Smith, 1986; Vane and Botting, 1998). COX enzymes are known for their ability to exert their cyclooxygenase activity through cyclization of arachidonic acid and the addition of the 15-hydroxyperoxy group for the formation of prostaglandin G_2 (Vane and Botting, 1998). This hydroxyperoxy moiety of prostaglandin G_2 is then reduced to the hydroxyl group of peroxidase H_2. This reduction is achieved through the activity of a peroxidase enzyme that utilizes a wide range of compounds to provide the required electron pair. The activities of both cyclooxygenases and hydroperoxidases are encompassed within the same protein dimer (Vane and Botting; 1998). The enzymatic family of cyclooxygenases is a key mediator in inflammatory responses, since they are involved in the synthesis of prostaglandins (PGs), a group of eicosanoid lipid compounds, from arachidonic acid. Prostaglandins, such as prostaglandin E_2 (PGE_2), are known for their ability to induce inflammation (Mizushima, 2010; Kawahara *et al*., 2015).

It is currently known that the COX enzyme family comprises two cyclooxygenase isoforms, termed COX-1 and COX-2. The COX-1 and COX-2 homodimers in humans have a length of 576 and 581 amino acids, respectively. Three high-mannose oligosaccharides are contained in both isoforms of COX, with one of them being responsible for the facilitation of protein folding. A fourth oligosaccharide is present only in the cyclooxygenase 2 isoforms, and its role lies in regulating its degradation (Rouzer and Marnett, 2009). The three-dimensional structure of cyclooxygenases was determined back in 1994 by Picot and their team, providing new insights regarding the actions of cyclooxygenase inhibitors.

Each subunit of the homodimer comprises three domains, including an epidermal growth factor domain encompassing residues 34 to 72, a membrane binding domain encompassing residues 73 to 116, and a catalytic domain that accommodates the bulk of the enzyme, with the heme prosthetic group containing the active sites for both peroxidase and cyclooxygenase on either side (Smith *et al*., 2000; Garavito *et al*., 2002; Rouzer and Marnett, 2003; Mbonye *et al*., 2008). COX-1 is often mentioned by scientists as a 'constitutive isoform' and for the most part it can be readily detected in most tissues under normal conditions. COX-2, on the other hand, is often mentioned as the 'inducible isoform' of COX, it is normally undetectable in most human tissues under basal conditions and its synthesis is believed to be mainly triggered by various pathological conditions. Various studies have also reported the presence of COX-2 in healthy tissues, particularly the parenchymal cells of the kidneys, brain and the female reproductive system (Komhoff *et al*., 1997; Harris and Breyer, 2001; Yermakova and O'Banion, 2001; Sirois *et al*., 2004; Stavreus-Evers *et al*., 2005; Zidar *et al*., 2009).

Both known cyclooxygenase isoforms have been found to exert a variety of different effects in different body tissues through the synthesis of prostaglandins. From the very early stages of life, COX-1 expression is apparent in the decidual lining of the uterus, as well as in many fetal organs, including fetal hearts, brains, lungs and kidneys. COX-2 is also expressed in these organs, although at much lower levels (Gibb and Sun, 1996; eds. Vane *et al*., 2015). Prostaglandin synthesis triggered through the expression of COX-1 is also responsible for the overall maintenance of a healthy gestation (Trautman *et al*., 1996). Expression of both COX isoforms occurs in the epithelium of the uterus at different times in early gestation, and might potentially be involved in ovular implantation and for the requisite angiogenesis for the development of the placenta (Chakraborty *et al*., 1996). Vasodilator prostaglandins are responsible for the regulation of kidney function in patients presenting with renal insufficiency, liver cirrhosis, and even congestive heart failure. The synthesis or prostacyclin and PGE_2 is mainly triggered by COX-1, although trace levels of COX-2 mRNAs have also been detected (Harris *et al*., 1994). Neurons of the brain have also been shown to express cyclooxygenase 1, although its presence is most abundant in the region of the forebrain. An explanation for that is the involvement of prostaglandins in

various brain functions, including sensory processing and the regulation of the autonomous nervous system (Yamagata *et al*., 1993; Breder *et al*., 1996). Trace amounts of COX-2 and its consecutive mRNAs have been reported in the forebrain under basal conditions, whereas higher levels have been detected in brain tissue and glial cell cultures, following exposure to lipopolysaccharide and interleukin-1 (Yamagata *et al*., 1993; Breder *et al*., 1995; Cao *et al*., 1995; Breder and Saper, 1996; Cao *et al*., 1996). Intense stimulation of the hippocampal neurons might lead to seizures, but at the same time stimulates the synthesis of COX-2 mRNA (Marcheselli and Bazan, 1996). Acute stress has been shown to induce an increase in COX-2 levels in the cerebral cortex (Yamagata *et al*., 1993). PGE_2 and prostacyclin also exert a profound effect on the gastrointestinal tract, as they have the ability to prevent gastric ulceration through reduction of gastric acid secretion and the process of vasodilation on gastric mucosa vessels. Moreover, the secretion of the viscous mucus that protects the gastrointestinal tract, is stimulated by prostanoids. These prostanoids are also responsible for the synthesis of gastric fluid and duodenal bicarbonate (Whittle and Vane, 1987). In humans, amongst other species, the majority of these protective prostaglandins are synthesized by the cyclooxygenase 1 isoform, although trace amounts of COX-2 have also been reported (Kargman *et al*., 1996). Bacterial infections of the gastric mucosa can also stimulate the synthesis of COX-2 (Whittle and Vane, 1987).

Prostaglandins, including PGE_2, have been shown to play a variety of other roles within the body. These roles include the inhibition of cellular apoptosis, as well as the stimulation of cellular growth, angiogenesis and cancer cell metastasis (Rolland *et al*., 1980; Tsujii *et al*., 1998; Hoshino *et al*., 2003). Interestingly enough, the synthesis of COX-2 can be induced by p53, mediated by Ras/Raf/ERK cascade activation. COX-2 enzymes have been found to neutralize cellular stresses that either activate or are activated by p53. These findings underline a novel potential role for COX-2 in the reduction of cytotoxicity responsible for the activation of p53 (Han *et al*., 2002). In opposition to that, COX-2 enzyme has been reported by scientists to be overexpressed in a variety of cancer cells and tissues (Eberhart *et al*., 1994; Ristimäki *et al*., 1997; Mizushima, 2010). As a result, cyclooxygenase inhibition by nonsteroidal anti-inflammatory

drugs was initially believed to be solely responsible for their anticancer activity. However, current knowledge suggests that the therapeutic and preventive effects of NSAIDs against cancer can also be achieved through mechanisms independent of COX enzymes. Sulindac sulfone, a chemical metabolite of the nonsteroidal anti-inflammatory drug sulindac, has been previously reported to display anti-cancer effects both *in vitro* and *in vivo* without causing inhibition of COX enzyme activity (Piazza *et al*., 1997; Reddy *et al*., 1999; Mizushima, 2010). Furthermore, studies have reported that NSAIDs were able to induce cell apoptosis and inhibit cell growth in COX-null fibroblasts and cancer cells that did not express members of COX enzyme family (Hanif *et al*., 1996; Zhang *et al*., 1999; Mizushima, 2010). Therefore, the assessment of those COX enzyme-independent mechanisms responsible for the chemopreventive effect of NSAIDs is essential for the development of novel NSAIDs with increased effectiveness against cancer (Mizushima, 2010).

1.3 Examples of NSAIDs

1.3.a Aspirin

Aspirin, also known as acetylsalicylic acid, is the most well-known antipyretic and analgesic drug globally. It is a salicylate, but it is also considered as a nonsteroidal anti-inflammatory drug (LiverTox, n. d.). Aspirin is classified as a class III drug on the Biopharmaceutics Classification System (BCS), meaning that it is characterized by high solubility and low drug permeability, with the absorption of aspirin being its rate limiting step (Benet, 2013; Najafzadeh *et al*., 2016). Like all members of the NSAID class of drugs, salicylates inhibit the cyclooxygenase proteins COX-1 and COX-2, causing a subsequent decrease in the synthesis of prostaglandins, which mediate both pain and inflammatory processes. In contrast with other NSAIDs, aspirin inhibits the cyclooxygenase 1 isoform non-competitively and irreversibly, exerting a more prolonged and stronger effect than those observed in typical nonsteroidal anti-inflammatory medications (LiverTox, n. d.). Aspirin is also an antiplatelet drug, with its profound inhibitory effects lasting for the entire lifetime of platelets. These lasting and irreversible effects of aspirin on COX-1 in cells of the gastric epithelia are responsible for the adverse side effects

observed in the gastrointestinal system, including peptic ulceration and bleeding (LiverTox, n. d.).

Colds, headaches, toothaches, menstrual periods, arthritis, and joint and muscle aches caused by rheumatoid arthritis, osteoarthritis, or trauma, are some of the commonest conditions for which the administration of aspirin for therapeutic purposes is frequent. Higher, more continuous aspirin doses are often administered for treatment of Kawasaki disease, systemic lupus erythematosus, juvenile rheumatoid arthritis, rheumatoid arthritis, and acute rheumatic fever (LiverTox, n. d.). Aspirin also has a profound antipyretic activity and is used for management of fever. Administration of aspirin for the treatment of fever, however, is prohibited in young children and adolescents, due to the potential of aspirin to cause development of Reye syndrome (LiverTox, n. d.). The daily intake of aspirin at low doses of 81mg is used to reduce the risk of developing cardiovascular problems, including cerebrovascular and coronary disease. Aspirin is also used to prevent vascular reocclusion following coronary revascularization or the placement of stents (LiverTox, n. d.). Aspirin is currently used as an off-the-counter medication, it can be obtained in a large number of generic formulations, either alone or in combination with other pain analgesics, cough and cold medications, or antacid substances. A typical intake dose of aspirin is approximately 330 to 660mg every 4 to 6 hours. A single, daily dose of 81mg of aspirin is used for its antiplatelet effects in order to prevent complications in patients presenting with atherosclerosis. Common brand names for aspirin alone or in combination with other agents include Bayer's Aspirin, Alka Seltzer, Ascriptin, and Anacin, among others (LiverTox, n. d.).

1.3.b Ibuprofen

Ibuprofen is a propionic acid derivative, and the member of the nonsteroidal anti-inflammatory drug family that is most frequently prescribed and most commonly used (LiverTox, n. d.). It is classified as a class II drug on the Biopharmaceutics Classification System, meaning that it has a high permeability, but a very low water solubility that consequently limits its total bioavailability within the body (Benet, 2013; Najafzadeh *et al*., 2016). Much like aspirin, ibuprofen is a non-selective NSAID that inhibits the action of both COX-1 and COX-2 enzymes.

Despite the fact that its anti-inflammatory activity is somewhat weaker, compared to other typical NSAIDs, ibuprofen is known to have profound antipyretic and analgesic properties that explain its frequent use (LiverTox, n. d.). Unwanted side effects of frequent ibuprofen intake include abdominal discomfort, nausea, vomiting, diarrhea and hypersensitivity reactions, although these side effects are less severe than those caused by aspirin (LiverTox, n. d.).

Ibuprofen is administered for mild-to-moderate cases of joint pain and arthritis deriving from trauma, rheumatoid arthritis or osteoarthritis. Ibuprofen is also effective in the treatment of other forms of pain, such as headaches and dysmenorrhea (LiverTox, n. d.). Ibuprofen is supplied in a tablet form, with a potency range between 200 and 800mg. The usual daily intake dose of ibuprofen ranges from 400 to 800mg, three times per day. Ibuprofen has poor water solubility but good oral absorption, with a serum half-life of approximately 2 hours. It is completely eliminated through normal body metabolism within 24 hours after the last dose (LiverTox, n. d.). In opposition to aspirin, pediatric formulations ibuprofen are also available. Advil, Motrin, and Nuprin are common brand names under which ibuprofen is frequently sold. Ibuprofen is also found in many combination formulations for the treatment of headaches, allergies, dysmenorrhea, and upper respiratory tract symptoms among others (LiverTox, n. d.).

1.3.c Naproxen

Naproxen is another propionic acid derivative, similar to ibuprofen, ketoprofen, and fenoprofen. The anti-inflammatory and analgesic properties of naproxen are achieved through the non-selective inhibition of cyclooxygenases 1 and 2, therefore resulting in reductions in prostaglandin synthesis (LiverTox, n. d.). Similar to ibuprofen, naproxen has a profound anti-inflammatory, analgesic and antipyretic activity. Naproxen, however, has a longer drug half-life compared to other nonsteroidal anti-inflammatory drugs (LiverTox, n. d.). Common side effects of naproxen use include headaches, nausea, vomiting, a feeling of abdominal discomfort, and hypersensitivity reactions. Other uncommon but serious side effects associated with frequent intake of naproxen include gastric ulceration and bleeding, renal dysfunction and failure, and a risk of developing cardiovascular disease (LiverTox, n. d.).

Naproxen is commonly used for treatment of mild-to-moderate pain resulting from different causes including trauma, headaches, dysmenorrhea, tendonitis, dysmenorrhea, and various forms of arthritis including rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis (LiverTox, n. d.). Naproxen is available as tablets, capsules and oral formulations, with a potency range between 200 and 550mg. It is sold under multiple commercial names including: Naprosyn, Aleve, and Naxen, and its typical dose ranges between 250 and 500mg, administered twice a day through oral administration (LiverTox, n. d.).

1.3.d Celecoxib

Celecoxib is a commonly used nonsteroidal anti-inflammatory drug and a selective COX-2 enzyme inhibitor. As its name suggests, celecoxib can prevent the synthesis of the pro-inflammatory prostaglandins through its relative COX-2 specificity (LiverTox, n. d.). This profound selectivity of celecoxib against cyclooxygenase 2 is believed to be responsible for conferring a decreased risk of adverse gastrointestinal symptoms compared to non-selective NSAIDs that inhibit the activity of both COX-1 and COX-2 isoforms (LiverTox, n. d.).

Administration of the COX-2 selective NSAID celecoxib is indicated for treatment of various forms of arthritis, including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis-derived chronic arthritis, and ankylosing spondylitis. Administration of celecoxib has also received approval for use in acute pain deriving from trauma and musculoskeletal conditions (LiverTox, n. d.). Celecoxib is also used to prevent the development of adenomatous polyposis, due to the prominent role of COX-2 in adenomatous polyp formation. Celecoxib is available under the commercial name Celebrex and it is prescribed as capsules with a potency range of 50 to 400mg. Administration of Celebrex can be done over the course of several weeks or it can be long-term. Recommended doses of celecoxib vary based on the pathological condition, with the standard dose for adults presenting with arthritis being between 100 to 200mg twice per day (LiverTox, n. d.). Side effects associated with frequent intake of celecoxib are similar to those caused by other NSAIDs and include headaches, rash, nausea, vomiting, diarrhea, abdominal discomfort, hypersensitivity reactions and the development of peripheral edema (LiverTox, n. d.).

1.4 Cancer

Cancer is a potentially fatal pathological condition characterized by uncontrolled cellular division in the body. Cancer is a disease group that encompasses more than 100 different diseases that develop gradually over time (NIH Curriculum Supplement Series, 2007). Cancer can develop in virtually any tissue within the human body, with different types of cancer demonstrating their own, unique features. Despite the fact that each type of cancer has these unique characteristics, the basis of cancer development remains for the most part unchanged in all different forms of the disease (NIH Curriculum Supplement Series, 2007).

Cancer development is initiated when a body cell deviates from normal cellular division and begins to proliferate uncontrollably. As a result, all descendant cells of this first, ancestral cell also display abnormal cell multiplication (NIH Curriculum Supplement Series, 2007). A mass of cells, formed of these abnormal cells is being referred to as a 'tumor'. Tumors may remain static within the tissue in which they initially manifested, in which case they are termed '*in situ* cancers', or they may start to invade normal adjacent tissues, in which case they are termed '*invasive*'. Invasive tumors are malignant, and tumor metastases in both nearby and distant tissues are likely to develop when cells deriving from a malignant tumor reach the bloodstream or the lymph nodes (NIH Curriculum Supplement Series, 2007). Tumors pose a serious threat to an individual's life when their abnormal development disrupts the structure and function of vital tissues and organs of an individual (NIH Curriculum Supplement Series, 2007).

Cancer is a multistep pathological condition that develops gradually over time as a prolonged and complex chain of changes to an individual's genetic material, and not all at once. Each change that occurs allows cells in their precancerous, non-malignant state to acquire some of the traits that are collectively responsible for the malignant growth of tumors (NIH Curriculum Supplement Series, 2007). Cancer development is normally caused by deviations in function in two different gene categories. These genes are normally involved in regulating the cell cycle, a process by which cells increase in size and, subsequently, number. The first category of genes is called 'proto-oncogenes', and their role lies in encouraging

cell division. The second category of genes is called ‘tumor suppressor genes’, and they’re responsible for the regulation and maintenance of cell division at normal levels (NIH Curriculum Supplement Series, 2007). When proto-oncogenes acquire a mutation, they are consequently transformed into oncogenes, which is a family of genes that triggers the excessive multiplication of cells. Mutated tumor suppressor genes, on the other hand, are known to completely lose their function, resulting in a total loss of their regulatory role that prevents uncontrolled cell division within the cell cycle. Mutations in both of these two gene categories are collectively responsible for the excessive and unregulated cellular multiplication that is observed in human cancer (NIH Curriculum Supplement Series, 2007).

1.5 Nanotechnology

Nanotechnology is a term often used to describe the manufacturing and manipulation of materials and structures presenting with at least one dimension with sizes ranging between 1-100nm (Laurent *et al*., 2008; Farokhzad and Langer, 2009). Sizes greater than 100nm and up until 1000nm have also been reported with regards to nanotechnology. Nanomaterials can be divided into 0D, 1D, 2D and 3D materials, based on their shape and the number of dimensions with sizes that fall within the 1-100nm range. In 0D nanomaterials, all three dimensions fall within the nanoscale, whereas 3D nanomaterials have no dimensions with a size between 1-100nm. 1D and 2D materials exhibit one and two dimensions in the nanoscale, respectively (Malhotra and Ali, 2017). Nanoparticles are of great interest to the field of modern medicine, since they present with unique characteristics and properties that are often absent in the macro scale. These novel features are attributed to nanoparticles due to their much smaller size and different shape. Such attributes can be thermal, optical, electronic, magnetic or mechanical (Laurent *et al*., 2008). For example, metallic nanoparticles showcase a higher thermal conductivity, compared to solid-form fluids. Moreover, nanoparticles have a higher elastic modulus and increased mechanical strength than their bulk counterparts, and often exhibit different excitation and emission wavelengths (Khan *et al*., 2019). Classification of nanoparticles is often done on the basis of their size, morphology and chemical properties. Characteristic classes of nanoparticles include carbon-based nanoparticles, polymers, metallic

nanoparticles, ceramics, semiconductors, and lipid-based nanoparticles (Khan *et al*., 2019).

The use of nanotechnology has brought about a revolutionalization regarding the synthesis of effective delivery systems that enhance the pharmacokinetics and pharmacodynamics of standard chemotherapy drugs. Even so, these innovative therapeutics are still highly dependent on active pharmaceutical ingredients that are costly and highly toxic, thus limiting their potential for widespread application (Tan *et al*., 2010). In this sense, nonsteroidal anti-inflammatory drugs commonly associated with chemoprevention, are also potentially involved in cancer ablation (Cuzick *et al*., 2009; Cronin-Fenton *et al*., 2010; Hossain *et al*., 2012). More specifically, various nonsteroidal anti-inflammatory drugs, such as aspirin (Harris *et al*., 2002; Zhang *et al*., 2005; Burn *et al*., 2011; Brasky *et al*., 2011; Harris *et al*., 2011; Hossain *et al*., 2012; Cook *et al*., 2013; Liu *et al*., 2013; Mansouri *et al*., 2013), celecoxib (Koki and Masferrer, 2002) and ibuprofen have been pinpointed for their potential chemotherapeutic activity (Harris *et al*., 2002; Zhang *et al*., 2005; Cronin-Fenton *et al*., 2010; Harris *et al*., 2011; Coghill *et al*., 2012; Liu *et al*., 2013). Nonetheless, the need to reduce the toxicity of NSAIDs to the gastrointestinal tract (Prakash *et al*., 2012) and first pass metabolism is of great importance. An increase in the biological availability of NSAIDs on the target cancer tissues is also deemed crucial (Boelsterli *et al*., 1995). To achieve this, scientists have since developed a wide range of different delivery vectors for the successful delivery of anticancer drugs, including liposomes (Tan *et al*., 2010), silica nanoparticles (Li *et al*., 2013), cell-penetrating peptides (Nakase *et al*., 2012) and polymeric micelles (Otsuka *et al*., 2003; Rössler *et al*., 2012).

2.0 NSAIDs in Cancer Prevention & Treatment

Over the years, the administration of nonsteroidal anti-inflammatory drugs for the prevention and/or treatment of cancer has been extensively studied as a novel treatment plan of great potential and promise. The majority of studies using NSAIDs have focused on their effects on the gastrointestinal tract. Examples of the most extensively studied cancers include gastric, colorectal, and esophageal cancer among others. Mansouri *et al*. (2013) studied the potential involvement of aspirin, statins and ACE-inhibitors on survival improvement and total recurrence reduction in patients presenting with cases of colorectal neoplasia.

A clinical trial study by Cook *et al*. (2013) investigated the correlation between cancer development and long-term, low-dose aspirin intake and placebo drugs in healthy females. Their results indicate the absence of extended effects of aspirin with regards to both breast and lung cancer. Colorectal cancer incidence, however, was found to be lower in females from the aspirin group. The side effects of aspirin, including gastrointestinal ulceration and bleeding, were apparent in this study (Cook *et al*., 2013). The use of NSAIDs has been uniformly found to cause polyp regression and prevent the development of new polyps, whereas aspirin intake has been documented to cause a reduction in the risk of adenoma recurrence in patients with sporadic adenomas. The effect of aspirin intake on cancer risk in carriers of hereditary colorectal cancer was investigated by Burn *et al*. (2011) within the context of the Colorectal Adenoma/Carcinoma Prevention Program (CaPP) randomized control trial. A daily dose of 600mg of aspirin for a mean of 25 months was reported to reduce cancer incidence in hereditary colon cancer carriers within a 58-month timeframe (Burn *et al*., 2011).

The use of aspirin and NSAIDs has also been shown to prevent cancer development in various organs. Long-term use of nonsteroidal anti-inflammatory drugs among women suggests a chemopreventive effect on colorectal neoplasia, as well as a noteworthy reduction in overall colorectal cancer mortality in post-menopausal females (Coghill *et al*., 2012). *In vitro* studies have shown that the use of NSAIDs might also be a useful tool in assessing the grade of malignancy in prostate cancer patients through the expression of the NSAID-activated gene 1 (NAG-1) (Kawahara *et al*., 2010). NAG-1, a transforming growth factor β

superfamily protein, is known to exhibit both proapoptotic and antitumorigenic properties, and its expression has been found to be significantly higher in malignant prostate and other tissues, compared to normal (Iczkowski and Pantazis, 2003; Chan and Detering, 2013). Celecoxib, a COX-2 selective NSAID, can induce apoptosis in both PC3 and LNCaP prostate cancer cell lines in a COX-2 independent manner. Celecoxib-induced apoptosis can be achieved through Akt inhibition and caspase-3 activation and through arrest at the G1 phase of the cell cycle (Hsu *et al*., 2000; Kulp *et al*., 2004; Patel *et al*., 2005).

In 2013, Kang *et al*. studied the effects of epigallocatechin-3-gallate (EGCG), a polyphenolic constituent of green tea with anti-inflammatory properties, and concluded that it was able to upregulate the expression of NAG-1 in an ataxia-telangiectasia mutated (ATM)/p53 gene-dependent mechanism. According to the findings of this study, the expression of NAG-1 was transcriptionally induced during EGCG-induced apoptosis of head and neck squamous cell carcinoma (HNSCC), therefore suggesting the presence of an additional mechanism for EGCG-related apoptosis (Kang *et al*., 2013). A meta-analysis of twenty-one studies carried out by Tian *et al*. (2010) suggested a protective effect of both aspirin and non-aspirin based NSAIDs against gastric carcinomas. Lastly, there are various studies that suggest a chemopreventive effect of aspirin and other NSAIDs, such as ketorolac, against breast cancer development and relapse (Brasky *et al*., 2011; Retsky *et al*., 2012). Data regarding the use of NSAIDs and the development of renal cell carcinoma (RCC) is inconsistent. In a meta-analysis by Liu *et al*. (2013), there was no statistically significant correlation between the intake of both aspirin and non-aspirin NSAIDs and RCC risk. There were, however, suggestions of an elevated RCC risk in subjects <63 years with frequent use of NSAIDs and a reduced risk in subjects ≥63 years with frequent aspirin intake.

A case control study of nonsteroidal anti-inflammatory drugs was conducted by Harris *et al.* (2002). Participants to this study included 489 test subjects presenting with lung cancer and 978 control subjects. All control subjects who participated in this study were heavy smokers. Control subject selection was done purposely in order to evaluate the effect of nonsteroidal anti-inflammatory drugs, such as aspirin, ibuprofen and prescription NSAIDs, on tobacco carcinogenesis (Harris *et*

al., 2002). Age, gender and pack-years of cigarette smoking of subjects were also taken into consideration as matching characteristics. Their results suggested that daily NSAID intake for a minimum of two years resulted in a total reduction of approximately 68% in the risk of lung cancer development. An inverse correlation of increasing daily doses of NSAIDs with a decreasing trend of tumor carcinogenesis was also apparent in this study (Harris *et al*., 2002). Results of this study combined with the current molecular evidence suggests that daily intake of nonsteroidal anti-inflammatory drugs might have a chemopreventive effect on tobacco carcinogenesis through a COX-2 blockade-dependent mechanism (Harris *et al*., 2002).

Apart from aspirin and ibuprofen, other nonsteroidal anti-inflammatory drugs have shown a very promising chemotherapeutic and chemopreventive potential. A nested-case control study by Sharpe and colleagues (2000) aimed to investigate the effects of nonsteroidal anti-inflammatory drugs on cases of invasive breast cancer. The team was unable to divide the NSAIDs into non-selective and COX-2 selective, as it was still unclear at the time whether it was COX-1, COX-2 or both COX enzymes that were involved in tissue carcinogenesis (Sharpe *et al*., 2000). A total of 5882 cases of invasive breast cancer and 23.517 healthy controls was used during in this investigation. Breast cancer patients were matched to healthy females based on their age and the time of sampling. Findings of this case-control study implied the existence of reduced risk for the development of breast cancer, following the increased and extended use of NSAIDs over the course of 2 to 5 and 6 to 10 years prior to diagnosis. NSAID exposure was able to significantly reduce the risk of patients presenting with large breast tumors or distant metastases (Sharpe *et al*., 2000). However, Sharpe and colleagues (2000) suggested that the use of NSAIDs in modern oncology might have a greater potential with regards to chemotherapy than to chemoprevention: NSAIDs might be more effective in the prevention and delay of post-diagnostic tumor metastases, compared to primary tumor development.

In 2005, Zhang and their team tested 7.009 females presenting with female cancer and 3.622 control cases as an extension of a previous hospital-based cased control study in the US. This case-control study focused on evaluating the association between the regular intake of NSAIDs and the risk of breast cancer

development. Various classes of nonsteroidal anti-inflammatory drugs were assessed in this study, including salicylates (e.g. aspirin), propionic acids (e.g. ibuprofen), and selective COX-2 inhibitors (e.g. celecoxib) (Zhang *et al*., 2005). Findings of this study suggest a statistically significant inverse correlation between the regular use of NSAIDs and breast cancer risk, which appeared to be stronger in postmenopausal females (Zhang *et al*., 2005). Interestingly enough, the class of NSAID used or the hormone receptor status of the tumor were not found to have a significant effect on the anticancer activity of the drugs (Zhang *et al*., 2005).

In another case control study published in 2011, Harris *et al*. compared the effect of selective and non-selective COX-2 inhibitors on 611 breast cancer patients. These incident cases were compared to 615 healthy volunteers. Breast cancer patients and cancer-free controls were matched at a 2:1 ratio. Matching criteria included age, race, and country of residence (Harris *et al*., 2011). Results of this case control study showed significant reductions to the risk of breast cancer development for selective COX-2 inhibitors such as celecoxib, as well as for regular aspirin, ibuprofen, and naproxen. This preventive effect of NSAIDs was apparent in both pre- and postmenopausal females, as well as in women with a positive or negative family history, women with estrogen receptor positive or negative tumors, and women with HER-2/neu positive or negative tumors (Harris *et al*., 2011). Other nonsteroidal anti-inflammatory drugs, including acetaminophen and low dose aspirin, were found to have no noteworthy effects to the risk of breast cancer development (Harris *et al*., 2011). Selective COX-2 inhibitors celecoxib and rofecoxib reportedly showcased a significant reduction of approximately 71% in the risk of breast cancer development, therefore underlining their strong anti-cancer potential (Harris *et al*., 2011).

Another paper by Ashok *et al*. (2011) reported similar findings. Ashok and their team conducted a large case-control study that lasted for three years, between 2003 and 2006. A total of 18.368 cases of incident breast cancer was identified using the Ingenix/Lab Rx insurance database and made use of during this study. Ashok and their team selected for random controls per case and matched them on age and time in the insurance database. Subjects were treated with non-selective NSAIDs and the COX-2 inhibitors celecoxib, rofecoxib and valdecoxib. In agreement with the findings of the previous case-control study by Harris *et al*.

(2011), Ashok and colleagues (2011) suggested that the intake of both non-selective and COX-2 selective NSAIDs exerted a preventive dose-response effect against breast cancer development. More specifically, the daily intake of a standard 200mg dose of celecoxib for a period of 12 months or greater was associated with a risk decrease of 16% with regards to breast cancer development. The greatest reduction in breast cancer risk was attributed to the daily intake of rofecoxib over the course of two years or more. Rofecoxib exposure was shown to cause a reduction of 46% in the risk of breast cancer. As for non-specific NSAIDs, the greatest risk reduction was observed in breast cancer patients with a daily income of the drugs for 2 to 3 years prior to their diagnosis. The use of acetaminophen in this study did not yield a significant chemopreventive effect against breast cancer (Ashok *et al*., 2011).

The antitumor effect of NSAIDs has also yielded promising results when administrated in combination with other pharmaceuticals. In 2009, Srinivas and Feldman tested whether treatment of patients with early recurrent prostate cancer could successfully delay the growth and progression of the disease. For this paper, Srinivas and Feldman enrolled 21 patients in total. Subjects in this study presented with biochemical relapse, following local prostate cancer therapy. Men were treated with high doses of the nonsteroidal anti-inflammatory drug naproxen and the Vitamin D active metabolite calcitriol (Srinivas and Feldman, 2009; Christakos *et al*., 2010). Calcitriol was administrated once a week, whereas naproxen was administrated twice daily. The doses of administration were 45µg for calcitriol and 375mg for naproxen. Following the administration of the drugs, patients were subject to evaluation of their prostate specific antigen (PSA) levels and imaging studies. PSA is considered to be a diagnostic biomarker, and high levels of PSA are often indicative of the presence of conditions of the prostate, although high levels of PSA are not cancer-specific (Stephan *et al*., 2014; Cabarkapa *et al*., 2016). Out of 21 prostate cancer patients tested, four of them reportedly met criteria for tumor progression, and their PSA doubling time (PSADT) decreased despite them being on combination therapy. Fourteen prostate cancer patients, however, showcased significant increases in the doubling times for PSA compared to baseline, therefore suggesting that combination therapy of naproxen and calcitriol is able to prolong the doubling time

of the prostate specific antigen and may consequently delay the onset and development of prostate cancer (Srinivas and Feldman, 2009).

Naproxen has also shown interesting results against subjects presenting with bladder cancer within a laboratory environment. Female lab rats were treated Lubet and colleagues (2015) with hydroxybutyl(butyl)nitrosamine (OH-BBN), a urinary bladder–specific carcinogen, for a total duration of 8 weeks. Bladder cancer-presenting rats were then given 40mg/kg of body weight naproxen or 4mg/kg of body weight omeprazole, a proton pump inhibitor (Lubet *et al*., 2015). Lab rats were treated solely with naproxen, omeprazole, or with a combination of the two, two weeks following their final exposure to OH-BBN. A total of 96% of rats treated with OH-BBN developed cancer of the urinary bladder (Lubet *et al*., 2015). Although single administration of omeprazole had no anticancer effect on 97% of the cancers, the use of naproxen alone or in combination with omeprazole had a chemopreventive effect of approximately 27% and 35%, respectively. In a separate, intermittent-dose study, Lubet and their team found that the use of naproxen either singly or in combination with a vehicle was able to significantly prevent the development of palpable cancers of the bladder, a finding that was not consistent with the administration of the vehicle alone (Lubet *et al*., 2015). The short-term administration of naproxen resulted in increases in apoptotic cell death of tumor cells, but had no apparent effect on cancer cell proliferation. Lubet *et al*. (2015) also attempted to establish protocols in order to overcome the adverse cardiovascular and gastrointestinal side effects of NSAIDs. Interestingly, the two different established protocols were not found to affect the chemopreventive activity of NSAIDs, setting a strong foundation for the application of naproxen in clinical trials for the treatment of bladder cancer (Lubet *et al*., 2015).

The use of naproxen has also exerted interesting effects against the MG-63 osteosarcoma cell line. Correia *et al*. (2014) examined the effect of various concentrations of naproxen on the MG-63 osteosarcoma cell line. Concentrations used in this paper include 0.03, 0.05, 0.1, 0.42, 0.83, and 1.67mg/ml for a total duration of 72 hours. The team performed a wide range of techniques in order to assess the anticancer activity of naproxen, including comet assay, MTT assay, TUNEL assay and acridine orange and monodansylcadaverine (MDC) staining (Correia *et al*., 2014). This paper further confirmed the potential of naproxen as a

chemotherapy and chemoprevention agent, as it exhibited a dose-dependent antiproliferative effect on MG-63 human osteosarcoma cells. This inhibition on cancer cell proliferation was present in all treatment groups, compared to their untreated counterpart (Correia *et al*., 2014). MG-63 osteosarcoma cells experienced noteworthy increases in the presence of apoptotic bodies and apoptotic cells, autophagic vacuoles and DNA toxicity. Correia and colleagues concluded that further studies are needed, but underlined the huge potential of naproxen for the treatment of osteosarcoma (Correia *et al*., 2014).

There is an alarming number of studies and papers that focus on evaluating the preventive and therapeutic activities of nonsteroidal anti-inflammatory drugs on various types of cancer. However, the use of NSAIDs as therapeutic agents for chemotherapy leaves normal cells and tissues of the body susceptible to the adverse side effects of NSAID intake. Gastrointestinal bleeding and ulceration, cardiovascular problems and NSAID-induced toxicity of the kidneys are only a few of the commonest adverse effects caused by the frequent intake of NSAIDs (Wongrakpanich *et al*., 2018).

During the last decade, the emerging field of nanotechnology has slowly started to take over modern oncology, focusing both on the unmasking of novel characteristics and attributes in current medicines and the development of state-of-the-art, innovative therapeutic agents. These promising features can be attributed to the unique characteristics of particles that fall within the nanoscale, including their smaller sizes, increased surface areas, as well as their unique chemical, optical and electrical properties (Verma and Stellacci, 2010; Khan *et al*., 2019).

3.0 Nano-NSAIDs: An Emerging Field in Modern Oncology

3.1 Suspensions of Nano-NSAIDs for Cancer Treatment

Despite not being so extensively studied when compared to their bulk counterpart, the use of NSAIDs in their nano-forms for the prevention and treatment of cancer seems very promising. In 2016, Najafzadeh and their team evaluated the effect of the bulk and nano forms of aspirin (ASP) and ibuprofen (IBU) on healthy individuals, and respiratory patients of various pathologies. For their research, whole blood was collected from healthy controls, as well as lung cancer, asthma and chronic obstructive pulmonary disease (COPD) patients. Lung cancer patients involved in this study should not have undergone chemotherapy or radiotherapy prior to this study (Najafzadeh *et al*., 2016). Anemia, previous occupational exposure to other nanoparticles, such as silica and asbestos, and the presence of any additional respiratory conditions was not taken into consideration as exclusion criteria. All patients in this study presenting with COPD were above 40 years of age, and all of them had a pack year smoking history of 10 years or more. A fixed ratio of forced expiratory volume (FEV1) to forced vital capacity (FVC) of less than 0.7 (<0.07) or the detection of emphysema were also present in all COPD patients (Najafzadeh *et al*., 2016). All asthma patients selected for this study were either non-smokers or had a history of smoking pack cigarettes for less than 10 years. Moreover, all of them presented with similar symptoms, including coughing, wheezing and intermittent breathlessness. Spirometry results always indicated the presence of variable or reversible airflow obstruction. Other conditions and findings, such as hay fever, allergic rhinitis, or high IgE with reduced eosinophils, were also possible (Najafzadeh *et al*., 2016).

For this experimental procedure, both aspirin and ibuprofen were initially milled, in order to obtain their bulk and nano forms. 3 and 4% (w/w) suspensions of aspirin and ibuprofen were prepared, respectively, and the z potential of these suspensions was calculated in triplicate (Najafzadeh *et al*., 2016). Particle size analysis for the suspensions was also carried out, using dynamic light scattering in triplicate. The particle size analysis for the bulk and nano forms of both NSAIDs was carried out both after milling and at the end of this experiment, in order to ensure that no significant differences had occurred with regards to particle size

(Najafzadeh *et al*., 2016). Laser diffraction spectroscopy was used to measure the particle size of the ASP and IBU bulk particles. Particle size analysis for the nano powders of NSAIDs was evaluated through dynamic light scattering (DLS). Both techniques were carried out using three samples of each drug. Images of the nanoparticles were taken using Transmission Electron Microscopy (TEM), in order to showcase the shape of the drug nanoparticles present in the suspensions (Najafzadeh *et al*., 2016). The patch-clamp technique was also carried out, using the bulk and nano forms of ASP, but not IBU, on lymphocytes from all four groups in order to test the differential effects of the bulk and nano powders on ion channel activity (Najafzadeh *et al*., 2016). Single cell gel electrophoresis/comet and cytokinesis block micronucleus assays were performed in order to assess the amount of DNA damage present in both untreated and NSAID-treated whole blood samples. Three different doses of 250, 500 and 1000μg/mL, for both forms of ASP and IBU, were used in these experiments in order to determine the optimal drug doses with no cellular toxicity. DNA damage in the single cell gel electrophoresis assay was expressed both as Olive tail moments and as % tail DNA. For the micronucleus assay, Najafzadeh *et al*. (2016) tested six different whole blood samples obtained from 5 healthy controls, 1 asthma patient, 1 COPD patient, and 3 lung cancer patients, whereas cell scoring was done using bright-field light microscopy. Statistical analysis involved the Kolmogorov-Smirnov test, as well as the Kruskal-Wallis, Mann-Whitney, and Wilcoxon Signed Rank tests for the single comet assay, and the chi-square test for the micronucleus assay, with a p value of 0.05 or less being considered statistically significant ($p \leq 0.05$). Lastly, confounding factors in this experiment were also expressed statistically, using the Mann-Whitney test and SPSS (Najafzadeh *et al*., 2016).

Results obtained through TEM and laser diffraction spectroscopy suggest that ASP crystals are of slightly larger size, compared to crystals of IBU. As for the patch-clamp technique, the nano form of aspirin reportedly presented with lower whole-cell currents and inhibited ion channel activity by approximately 20%, compared to its bulk counterpart (Najafzadeh *et al*., 2016). In addition to the nano-associated cytotoxicity reported in lymphocytic cells, nano ASP was also found to produce a more marked response than bulk ASP, a finding that has also been mentioned in previous research papers (Shang *et al*., 2014; Najafzadeh *et al*.,

2016). For the single cell gel electrophoresis and micronucleus assays, optimal doses with no cytotoxicity were reported to be 500 and 250μg/mL, respectively. Optimal drug doses were found to be the same for both aspirin and ibuprofen (Najafzadeh *et al*., 2016). Overall, results for the comet assay showed significant decreases in the amount of lymphocytic DNA damage in all four groups, following treatment with the nano forms of aspirin and ibuprofen, compared to their bulk counterparts. Comparison of cells treated with the nano form of aspirin to untreated lymphocytes also showed a total reduction in DNA damage, with the exception of the asthma group (Najafzadeh *et al*., 2016). Treatment with the nano form of ibuprofen, however, showed a decrease in DNA damage for the healthy control and lung cancer group, and an increase for the asthma and COPD group, compared to untreated lymphocytes. Moreover, treatment of samples with the bulk forms of both aspirin and ibuprofen resulted in an increase in lymphocyte DNA damage, compared to untreated lymphocytes, with the exception of the lung cancer group (Najafzadeh *et al*., 2016). As for the micronucleus assay, treatment with the bulk forms of aspirin and ibuprofen resulted in a decrease in the number of micronuclei in binucleated cells and non-divided, mononucleated cells, in lung cancer patients and healthy donors, compared to untreated lymphocytes. On the other hand, findings of this study suggest an increase in the total number of micronuclei in lymphocytes obtained from asthma and COPD patients, following treatment with both forms of aspirin and ibuprofen. However, a reduction in DNA damage was reported for all four groups, following treatment with nano aspirin and nano ibuprofen, compared to their bulk counterparts (Najafzadeh *et al*., 2016).

Overall, their results indicate that both aspirin and ibuprofen might act as potential treatments for lung cancer patients, with an obvious chemotherapeutic effect on lymphocytes. Interestingly enough, lymphocytic DNA damage is reported to be a good indicator of the presence of carcinogenesis in many parts of the human body, including the breasts, ovaries, and testicles among others. That is mostly due to the ability of lymphocytes to reach and access a variety of different tissues while traveling in peripheral blood (Sánchez *et al*., 2004; Sestakova *et al*., 2016). Their results also showcase that this therapeutic effect can be further amplified for the nano forms of these two NSAIDs, compared to bulk. This, however, might not be the case for other respiratory pathologies, such as asthma and chronic

obstructive pulmonary disease, thus indicating a promising specificity of nonsteroidal anti-inflammatory drugs with regards to tumor-associated DNA damage (Najafzadeh *et al*., 2016). Moreover, the differential chemotherapeutic and chemopreventive effects of other nano-NSAIDs, such as naproxen, celecoxib and diclofenac, have yet to be tested.

In a similar experimental approach to that of Najafzadeh and their team, Dandah *et al*. (2018) evaluated the anticancer effects of the bulk and nano forms of aspirin and ibuprofen, this time on patients presenting with cases of breast cancer. Once again, lymphocytes were selected as the cell of choice for this paper, since they have the ability to reflect DNA genotoxicity caused by both endogenous and exogenous factors, whether physical or chemical (Najafzadeh *et al*., 2012; Anderson *et al*., 2014).

For this research paper, the team collected whole blood from healthy females and breast cancer patients, after receiving informed consent. Following sample collection, suspensions of both aspirin and ibuprofen were prepared, and the suspensions were later milled in order to prepared the bulk and nano powders (Dandah *et al*., 2018). For aspirin, 3% w/w suspensions were prepared, whereas for ibuprofen, 4% w/w suspensions were prepared. All suspensions were prepared using solid loads of NSAIDs in the suspension medium. As with the previous paper, 250µg/mL of ibuprofen and 500µg/mL of aspirin were selected as optimal doses with no obvious cytotoxic effect on the lymphocytes of both breast cancer patients and controls (Dandah *et al*., 2018). Prior to being used for the comet assay, twenty stored whole blood samples, each obtained from breast cancer patients and healthy controls, were used for the preparation of blood suspensions. Whole blood was added to RPMI-1640 culture medium, and mixed with negative control solvent, positive control, and the previously mentioned NSAIDs, in bulk and nano forms (Dandah *et al*., 2018). The alkaline comet assay and the cytokinesis block micronucleus assay were then carried out in order to evaluate the total amount of DNA damage observed in lymphocytes. Cells in the comet assay were stained using ethidium bromide and scored through fluorescence microscopy. As for the micronucleus assay, freshly obtained blood, from each of five breast cancer patients and five healthy controls, was used in order to prepare 72-hour lymphocyte cultures under sterile conditions. Cytological scoring in the

micronucleus assay was done using various parameters including the number of mononucleated, binucleated, and multinucleated cells, as well as the nuclear division index (NDI) (Dandah *et al*., 2018). Other external factors, including smoking and ethnicity, were also taken into consideration. These factors, however, were not found to significantly affect the response to treatment in neither the control nor the patient groups (Dandah *et al*., 2018). Lymphocyte viability testing was carried out 30 minutes after treatment with ASP and IBU using the trypan blue viability test. Obtained results were statistically analyzed using Graphpad and SPSS (Dandah *et al*., 2018).

The percentages of females that were smokers were 35% for breast cancer patients and 11% for the healthy volunteers. However, neither smoking status nor ethnicity were found to cause statistically significant differences with regards to the findings of this study (Dandah *et al*., 2018). For the alkaline comet assay, the results showed that ibuprofen was able to reduce the amount of total DNA damage in lymphocytes of breast cancer patients. The reduction in DNA damage, however, was not found to be of statistical significance. Aspirin, on the other hand, was shown to cause a significant reduction in lymphocytic DNA damage for breast cancer patients. The findings of Dandah and their team seem to be in agreement with the results of previous studies in support of the chemopreventive and chemotherapeutic effect of NSAIDs. NSAIDs have been implied to prevent tumors, including breast cancer, and this preventive effect may be achieved through COX-inhibitory mechanisms and the expression of p53 and other tumor suppressor genes. However, samples collected from healthy female volunteers presented with weak increases in lymphocytic DNA damage, compared to untreated cells. These increases were not found to be of statistical significance. Neither aspirin nor ibuprofen was reported to showcase statistically significant differences between their bulk and nano formulations(Dandah *et al*., 2018) . As for the micronucleus assay, a general reduction in the total number of micronuclei was evident for both forms of aspirin and ibuprofen. Both NSAIDs were found to cause a reduction in the amount of DNA damage in lymphocytes obtained from females presenting with breast cancer. Aspirin, however, was found to give a statistically significant reduction in lymphocytic DNA damage in both assays, whereas ibuprofen showcased reductions in DNA damage, for both bulk and nano forms, which were

only statistically significant in the micronucleus assay. Dandah and their team suggested that this deviation might be due to differences in incubation times, with the incubation times for the comet and micronucleus assays being 30 minutes and 72 hours respectively. Their results were also in agreement with the idea that nanoparticles can cross nuclear membranes more effectively due to their much smaller sizes, compared to their bulk counterpart (Dandah *et al*., 2018).

3.2 Polymer-coated Nano-NSAIDs for Cancer Treatment

Not all studies focusing on the anticancer effects of NSAIDs have used bare nanoparticles in solutions. In a research by Kumar *et al*. (2016), the anticancer effects of the nonsteroidal anti-inflammatory drugs naproxen, ibuprofen and ketoprofen were examined *in vitro* using five different cancer cell lines. The cytotoxicity of naproxen against tumor cells has been studied on many cancers, including cancers of the bladder and the prostate (Srinivas and Feldman, 2009; Lubet *et al*., 2015). Moreover, naproxen has also been shown to have an inhibitory effect on osteosarcoma development (Correia *et al*., 2014)). This chemopreventive and chemotherapeutic potential of naproxen, however, has been implied to involve other mechanisms in addition to their anti-COX activity (Kim *et al*., 2014). NSAIDs used in this study were not only used in their raw form, but were also coated using three different polymeric stabilizers, in order to assess whether the incorporation of polymers could further enhance the anticancer activity of NSAIDs (Kumar *et al*., 2016).

For this experimental procedure, Kumar and their team used the following five lines of cancer cells: the human breast cancer cell line MCF-7, the human pancreatic cancer cell line MIA-PA-CA-2, the human colon cancer line HT-29, as well as the human leukemia and ovarian cancer cell lines Jurkat and A2780. 100mM stock solutions were prepared using precisely weighed drug quantities. Stock solutions were further diluted in order to produce solutions of lower drug concentrations. Later on, drug solutions were filtered and then added to aqueous solutions containing three different polymeric stabilizers. The polymers were used in the antisolvent for the encapsulation and inhibition of growth of NSAID nanoparticles. Stabilizers used in this study include polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) and hydroxypropyl methylcellulose (HPMC) (Kumar *et al*.,

2016). Following solution preparation, the size, zeta potential and polydispersity index (PDI) of the particles was measured at a wavelength of 633nm using dynamic light scattering. Field emission scanning electron microscopy (FESEM) technique was used to obtain information on particle size and the morphology of the samples. Three different techniques, Fourier-transform infrared (FTIR) spectroscopy, X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC) were also used in order to study the NSAID-polymer conjugates (Kumar *et al*., 2016). The solubility of the three NSAIDs used in this study was calculated by initially dissolving a known excess quantity of the NSAIDs in water and them processing them through magnetic stirring, centrifugation, filtering and dilution using known volumes of water (Kumar *et al*., 2016). A UV-visible spectrophotometer was used to evaluate the relative concentrations of the raw NSAIDs and their respective nanoformulated forms through optical density measurements. Samples were dissolved in ethanol and processed in order to assess the encapsulation efficiency (%EE) of the polymers. The %EE was calculated through measurements of the amount of NSAID present in each solution. A UV-vis spectrophotometer was used to record the absorption spectra of the solutions, whereas the concentrations of ethanol-dissolved NSAIDs were calculated from a calibration curve by serial dilution of a standard solution (Kumar *et al*., 2016). The weight of the drug that was dissolved in ethanol was divided by the total weight of the drug added initially and then multiplied by 100 in order to obtain the encapsulation efficiency of the polymeric stabilizers. The drug release profile of the samples was tested using a USP microprocessor dissolution test apparatus (Kumar *et al*., 2016). Kumar and their team cultured tumor cells of five different cells lines and proceeded to assess the viability of the cultured cells using the trypan blue dye viability test. Cells presenting with a viability higher than 97% (>97%) were later used for cytotoxicity studies. The antitumor effect of all three raw NSAIDs and their respective bare and polymer-conjugated NSAID nanoparticles on cell cultures was assessed using the sulforhodamine B (SRB) and MTT assays. Both the optical density for the SRB and the absorption values for the MTT assays were read at a wavelength of 540nm (Kumar *et al*., 2016).

In this paper, various NSAID concentrations ranging from 5 to 100mM were used for all three drugs in order to study the effects of drug concenctration on particle

size. The results of the dynamic light scattering technique suggest the presence of a concentration-dependent increase in drug particle size. More specifically, the size of particles was observed to increase in correlation with increasing NSAID concentrations in prepared solutions. It was apparent that the size of the NSAID nanoparticles inside the solutions was influenced by the presence of stabilizers inside the antisolvent (Kumar *et al*., 2016). Polymeric stabilizers were also detected to affect the size of nanoparticles in a concentration-dependent manner. This evident effect of the polymers can be achieved through their absorption on the surface of nanoparticles and prevention of nanoparticle aggregation. Overall, the absence of stabilizers inside the antisolvent was reported to result in larger nanoparticle sizes (Kumar *et al*., 2016). Results obtained through FTIR showed that the interaction between the NSAIDs and the polymeric stabilizers is established through weak van der Waal's forces, whereas the X-ray diffraction technique suggested similar crystal structures for both raw and polymer-conjugated nano NSAIDs. According to the findings obtained through DCS, the melting points of nanoformulated NSAIDs were somewhat similar and occasionally slightly lower than those of their raw counterpart, whereas their crystallinity was lower (Kumar *et al*., 2016).

With regards to drug solubility, raw NSAIDs showed decreased solubility compared to free NSAID nanoparticles. Moreover, the prepared nano NSAIDs showed lower solubility values than polymer-coated NSAID nanoparticles (Kumar *et al*., 2016). Drug release of nanoformulated drugs from the polymer inhibitor nanocapsules was also reported to be higher than both raw drugs and bare NSAID nanoparticles. That was the case for all NSAIDs used in this study. Out of the three polymer stabilizers, HPMC was reported to be the most efficient when it comes to increasing drug dissolution (Kumar *et al*., 2016).

The use of most nanoformulated NSAIDs in this study showcased a good antitumor activity against the Jurkat leukemia cell line. On the contrary, when compared to the standard chemotherapeutic drug doxorubicin, the same drugs had poor anticancer activity with regards to the other four cancer cell lines (Kumar *et al*., 2016). Naproxen had a better antitumor activity compared to ibuprofen and ketoprofen. Interestingly enough, naproxen was implied to have a strong anticancer effect against leukemia, even in its raw form. The GI50 value for the

standard chemotherapy drug doxorubicin was considerably higher than that of naproxen conjugated with HPMC or PVP. Naproxen in conjugates with HPMC and PVP had very good anti-leukemia activity at all concentrations, with their anticancer effects being constantly stronger than those of doxorubicin. Kumar *et al*. even reported that PVP-conjugated naproxen was two times stronger as a chemotherapy agent than the standard drug. A possible explanation for these results is the fact that a decrease in the size of naproxen is in correlation with an increase of its solubility, although Kumar and their team (2016) found it impossible to base the amplified anticancer effect of naproxen solely on its increased solubility in this study. Apart from its solubility, naproxen derivatization is also known to enhance its activity as a chemotherapeutic. Another advantage of NSAIDs in modern oncology is the fact that the inflexibly low GI50 value required of other chemotherapy agents, need not be applied for the use of NSAIDs as anticancer drugs (Kumar *et al*., 2016). The cytotoxicity of nano-NSAIDs can also be attributed to the unique properties that are associated with their small size, including nanoparticle shape, charge, and surface characteristics among others (Verma and Stellacci, 2010). Finally, the use of raw polymers was proven to have no anticancer effect against all five cancer cell lines, further confirming the antitumor effects of nonsteroidal anti-inflammatory drugs (Kumar *et al*., 2016).

This study suggests that naproxen has a noteworthy activity against leukemia, which can be further enhanced on the nano scale. Although the bulk forms of both ibuprofen and ketoprofen do not have any significant anti-leukemia effects when compared to common chemotherapy medications, such as doxorubicin, their anticancer effects appear to be increased using nanoformulations. These effects seem to be further enhanced when polymer stabilizers are used as nanoparticle coatings (Kumar *et al*., 2016). In fact, drug repositioning, i.e. the investigation of already approved medications for novel therapeutic applications, is praised by scientists as a cost- and time-efficient perspective in the development of chemotherapy medications. The concept of decreasing the size of particles of NSAIDs with poor water solubility seems very promising for the development of drugs with anticancer properties (Kumar *et al*., 2016).

3.3 The Use of Nano-NSAID Prodrugs in Cancer Treatment

The use of NSAID nanoprodrugs in cancer research has also yielded very promising results. In a research paper from 2011, Lee *et al*. demonstrated that poor solubility prodrug compounds, such as those derived from the acidic NSAID flufenamic acid (FA), can give rise to nanostructures characterized by great stability and noteworthy anticancer effects. The use of NSAID prodrugs has been studied thoroughly during the last decades, and its main objectives are to help ameliorate the adverse side effects induced upon the organism by the use of NSAIDs and mask the free carboxylic groups of NSAIDs through bioreversible bond formation, therefore increasing their availability within the organism (Tammara *et al*., 1993; Bonina *et al*., 2001; Chandrasekaran *et al*., 2006; Siskou *et al*., 2007; Velázquez *et al*., 2007). As mentioned above, such side effects include mucosal inflammation and ulceration within the gastrointestinal tract (Whittle, 2003; Jin, 2015; Wongrakpanich *et al*., 2018). Such side effects are caused by the inhibition of the COX-dependent synthesis of prostaglandin within the GI tract, as well as the inflammatory response caused by the free carboxylic groups present in NSAIDs (Dannhardt and Kiefer, 2001).

Flufenamic acid is an acidic NSAID that has been shown to exert its effect through a COX-inhibitory mechanism of action (van Antwerpen *et al*., 2007). Moreover, flufenamic acid can inhibit the formation of hypochlorous acid by myeloperoxidase (MPO), a heme-containing enzyme involved in the complex defense mechanism protecting the body against exogenous aggregations. It has also been shown to prevent the development of amyloid cardiomyopathy and senile systemic amyloidosis, along with several other NSAIDs (Furtmüller *et al*., 2000; Klabunde *et al*., 2000; Arnold *et al*., 2003).

In order to test the anticancer effects of FA, synthesized compounds were purified and confirmed through thin layer chromatography technique (TLC). Further detection and separation of prodrugs and their degradation products was done using high-performance liquid chromatography (HPLC) at a wavelength of 254nm. Ultraviolet (UV) light was used for the visualization of oxidized flufenamic acid derivatives (Lee *et al*., 2011). Monomeric derivatives of α-lipoic acid (ALA) with tetraethylene glycol (TEG) were synthesized and characterized according to the

protocol, followed by the subsequent synthesis and characterization of monomeric (FA-TEG-ALA) and dimeric (FA_2TEG) flufenamic acid derivatives. FA-TEG-OH was also synthesized and used for the identification of breakdown products of the hydrophobic FA-TEG-ALA and FA_2TEG prodrugs during hydrolytic enzyme degradation (Lee *et al*., 2011). Column chromatography technique was used for the purification of purification of both monomeric and dimeric FA derivatives, and the separation of the latter from FA-TEG-OH (Lee *et al*., 2011).

Flufenamic acid derivatives were mixed with the lipid-soluble antioxidant α-tocopherol in order to prepare FA nanoprodrugs. Similarly, an α-tocopherol control nanosphere was synthesized in the absence of flufenamic acid. The synthesis of nanoprodrugs containing coumarin 6, a hydrophobic fluorescent dye, was also carried out for means of cellular uptake visualization (Lee *et al*., 2011). Dynamic light scattering technique (DLS) was performed for measurement of the nanoprodrugs hydrodynamic size and size distribution. The mean diameter and polydispersity index of the particles was calculated in triplicate for each nanoprodrug preparation. For this paper, the team also calculated the error bar (SD) of three determinations (Lee *et al*., 2011). Nanoparticle tracking analysis (NTA) was carried out for nanoprodrug visualization, followed by assessment of flufenamic acid nanoprodrug stability and enzymatic degradation through hydrolysis. Long-term storage stability of the synthesized flufenamic acid nanoprodrugs was determined through measurement of the nanoprodrug size and the amount of intact prodrugs following storage at low temperature over the course of 8 weeks. Concentrations of the intact FA prodrugs were assessed by reversed phase high-performance liquid chromatography (RP-HPLC). The recovery yield of prodrugs was calculated by dividing the amount of prodrugs after incubation with the amount of prodrugs prior to incubation, and then multiplying the quotient by 100. Again, the error bar (SD) was calculated from triplicate determinations (Lee *et al*., 2011). Enzymatic hydrolysis of flufenamic acid nanoprodrugs was evaluated through suspension in phosphate buffered saline and esterase. Enzymatic hydrolysis of nanoprodrugs was confirmed through addition to acetonitrile and RP-HPLC analysis. Lastly, cellular uptake of fluorescently labelled nanoprodrugs was tested on a human primary glioblastoma cell line using fluorescence microscopy, and the effects of flufenamic acid treatment on cancer cell viability were assessed.

Glioma cells were also treated with the control sphere containing α-tocopherol only. Triplicate determinations were used for the calculation of the error bar (SD) (Lee *et al*., 2011).

The synthesis of flufenamic acid prodrugs and their subsequent conversion into nanosized prodrugs offers a number of advantages, all of which are attributed to the unique characteristics associated with the nano scale. Examples of unique characteristics associated with nanostructures include nanoparticle shape, charge, and increased surface areas, compared to bulk structures. These attributes are responsible for the enhanced therapeutic efficacies of nanodrugs (Huang *et al*., 2003; Heckert *et al*., 2008; Verma and Stellacci, 2010). Synthesis of stable nanoconstructs appears to be strongly dependent on their hydrophobicity. Hydrophobic compounds are known to form structures of greater stability, due to the strength of hydrophobic bonds between molecules. Hydrophobic structures express prolonged periods of stability in aqueous environments within the body, a characteristic that is caused by the strong hydrophobic interactions and the inherent insolubility of hydrophobic compounds (Chuang *et al*., 2008). The compactness and hydrophobicity of the structure decrease interactions with water within biological aqueous environments, therefore increasing the integrity of nanostructures (Lee *et al*., 2011).

Interestingly, the addition of α-tocopherol did not result in significant differences in size for FA_2TEG. FA-TEG-ALA monomers, on the other hand, showcased smaller sizes upon the addition of α-tocopherol. Lee and their team (2011) speculated that this could be due to the fact that the addition of α-tocopherol increased the total hydrophobicity of FA-TEG-ALA, whereas such increase was not detected for FA*2*TEG. Both monomeric and dimeric FA derivatives had similar small sizes in the presence of α-tocopherol. This size similarity is generally of great importance, as it eliminates all other contributing factors and allows the therapeutic efficacy of the prodrugs to be evaluated solely by the different prodrug molecules (Lee *et al*., 2011). As a result, FA derivatives were prepared in the presence of α-tocopherol (Lee *et al*., 2011).

Lee and their team reported that both FA nanoprodrugs expressed the same particle size prior and post-storage over the course of 8 weeks. Furthermore, the

recovery yields obtained for FA-TEG-ALA and FA_2TEG were 75% and 90%, respectively. It is implied that the increased hydrophobicity of FA_2TEG is responsible for the formation of structures of greater stability and compactness. This might subsequently reduce interactions with water in the biological aqueous environments, thus decreasing prodrug hydrolysis and maintaining their structural integrity (Lee *et al*., 2011). Both flufenamic acid derivatives were reported to be able to form stable prodrug nanostructures that are readily activated through enzymatic hydrolysis and can cause growth inhibition of tumor cells. Monomeric FA nanoprodrug derivatives, however, were found to demonstrate a more significant inhibition on glioma cell growth and induction of cancer cell death, compared to their dimeric counterpart. Moreover, both nanoprodrugs exhibited similar intracellular uptake profiles, with FA-TEG-ALA exerting a stronger effect that has been attributed to a higher parent drug concentration and a consequently higher enzymatic activation (Lee *et al*., 2011). Lastly, monomeric FA nanoprodrugs in this study showcased a stronger inhibition of cell growth compared to the free form of the NSAID, implying the existence of a nanoprodrug-specific mechanism of delivery (Lee *et al*., 2011).

3.4 The Use of NSAID Nanocarriers in Cancer Treatment

Last but not least, the use of various nanocarriers for the delivery of NSAIDs to the tumor site has received increasing attention throughout the years. The utilization of nanocarriers for the delivery of NSAIDs in modern oncology aims in overcoming the gut toxicity, first pass metabolism and limited bioavailability at the tumor microenvironment that is associated with these drugs (Boelsterli *et al*., 1995; Prakash *et al*., 2012). Examples of nanocarriers used for the delivery of nonsteroidal anti-inflammatory drugs for chemopreventive and chemotherapeutic purposes include micelles, silica nanoparticles and liposomes among others (Otsuka *et al*., 2003; Tan *et al*., 2010; Rössler *et al*., 2012; Li *et al*., 2013). According to a research paper from 2011, Nie *et al*. organized an experiment to test the incorporation of phospho-ibuprofen (P-I, MDC-917) into liposomal and micellar nanocarriers, and their subsequent utilization for the treatment of human colon adenocarcinoma. Phospho-ibuprofen is often being mentioned as a safer alternative to ibuprofen, since long-term use of ibuprofen has been associated with toxicities in the gastrointestinal and cardiovascular systems (Wolfe *et al*., 1999;

Hippisley-Cox and Coupland, 2005). The synthesis of phospho-ibuprofen through the addition of a diethylphospho-butanol moiety to the carboxylic acid group of ibuprofen via an ester link has previously been shown to successfully inhibit the growth of colon cancer cells in murine models, while showcasing minimal toxicity to healthy cells (Huang *et al*., 2011; Xie *et al*., 2011).

Nie and their team (2011) initially prepared liposome-encapsulated phospho-ibuprofen using the thin lipid film hydration method. The team dissolved phospho-ibuprofen, soy phosphatidylcholine (Soy-PC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)2000] ammonium salt (DSPE-PEG) in chloroform evaporated to a thin film, rehydrated using phosphate buffered saline gradually extruded using double polycarbonate membranes. Extensive dialysis against saline was used to separate the liposomal constructs from non-encapsulated ibuprofen. Empty liposomes were also prepared as a control (Nie *et al*., 2011). The amount of liposome-encapsulated phospho-ibuprofen was evaluated by high performance liquid chromatography. Preparation of micellar constructs was done through dropwise addition of dilute acetone solution of phospho-ibuprofen and poly(ethylene oxide-b-lactic acid) (PEO-b-PLA) into phosphate buffered saline. Acetone was removed from the mixture under conditions of reduced pressure. Extensive dialysis was again used for the removal of non-encapsulated ibuprofen and trace amounts of the organic solvent. Empty micelles were synthesized as a control (Nie *et al*., 2011). The amount of encapsulated p-ibuprofen was determined using high performance liquid chromatography. Transmission electron microscopy with negative staining of 1% uranyl acetate and 2% phosphotungstic acid was used to study the morphology of the synthesized nanocarriers of phospho-ibuprofen. Particle size determination of the liposomal and micellar carriers was done in triplicate using dynamic light scattering technique. Stability of the p-ibuprofen-encapsulating liposomes and micelles was evaluated both in cell culture medium and in the presence of purified esterase, using reversed phase HPLC and HPLC, respectively. Stability test were performed in triplicate (Nie *et al*., 2011).

Nie and colleagues (2011) cultured three different cell lines of human colon adenocarcinoma: HT-29, HCT116 and SW480. Cancer cell viability was evaluated using the MTT assay (Nie *et al*., 2011). Cells were exposed to various

concentrations of raw p-ibuprofen, hollow nanocarriers, and p-ibuprofen-loaded nanocarriers, ranging from 0 up to 200μM. Cells from the SW480 cell line were used to study the uptake of P-I-loaded nanocarriers in human colon cancer cells. Cancer cells were seeded and cultured in order to form a confluent cell monolayer. Cells of the monolayer were treated with 10μL of specified p-ibuprofen or p-ibuprofen-containing suspensions. Excess nanocarriers not taken up by the cells were washed away using phosphate buffered saline (Nie *et al*., 2011). Nanocarrier concentrations within the cancer cells were determined using high performance liquid chromatography. This experiment was performed in triplicate. Raw and liposomal p-ibuprofen were administered through intraperitoneal injection to female BALB/c mice at a dose of 400mg/kg of body weight. Mice were sacrificed 15 minutes post-injection, and their blood and internal organs were collected. Blood samples were centrifuged to obtain the plasma, whereas organs were rinsed in triplicate in dimethyl sulfoxide (DMSO) (Nie *et al*., 2011). For drug level analysis, Nie and their team (2011) added acetonitrile to the plasma samples, followed by subsequent mixing and centrifugation, and the supernatants were analysed using high performance liquid chromatography. Homogenization of the internal organs was done in phosphate buffered saline. Organs were later sonicated and extracted with acetonitrile. Subsequent steps for the determination of p-ibuprofen levels were the same as those applied to the plasma samples. Another, single dose of 100mg/kg of raw and liposomal p-ibuprofen was again administered through intraperitoneal injection to study and compare the pharmacokinetics of the drugs. Mice were sacrificed at designated time points post-injection, their plasma was obtained and immediately deproteinized through mixing with acetonitrile, and p-ibuprofen and its metabolic derivatives were analyzed by HPLC (Nie *et al*., 2011). Murine models were also used for the assessment of the in vivo anticancer efficacy of the drugs. Mice were initially inoculated with human colon cancer cells. When the volume of the tumor reached 200mm^3, test subjects were divided into three groups and treated with phosphate buffered saline, 100mg/kg raw p-ibuprofen per day, or 100mg/kg liposomal p-ibuprofen per day. Tumors were measured twice a week. After a treatment duration of 3 weeks, the mice were sacrificed and their tumors were excised and weighed. Statistical analysis of the results was carried out using one-way ANOVA

with Tukey's HSD *post hoc* tests. P values equal or less than 0.05 were considered to be statistically significant (Nie *et al*., 2011).

The partition coefficient for p-ibuprofen was much higher than that of plain ibuprofen, indicating that P-I has a higher hydrophobicity. This finding potentially explains why p-ibuprofen is more efficiently incorporated into liposomal and micellar constructs (Nie *et al*., 2011). Plain ibuprofen was reportedly unable to be loaded in nanocarriers, particularly liposomes, supporting the claim that the use of p-ibuprofen might be a more effective alternative in the use of NSAIDs in cancer treatment (Nie *et al*., 2011). Results obtained through transmission electron microscopy revealed that p-ibuprofen-containing liposomal constructs were able to maintain their lipid bilayer structure, expressing a hydrodynamic radius of approximately 209nm. Liposomes were negatively charged and had a negative z potential. Polymeric micelles containing p-ibuprofen presented with a denser structure and a particle size of around 78nm. Moreover, micelles were found to have a neutral charge and a negative z potential (Nie *et al*., 2011). Experiments carried out for this paper indicated that the uptake of p-ibuprofen was both time- and dose-dependent. Interestingly enough, micellar and liposomal constructs encapsulating p-ibuprofen experienced greater cellular uptake by cancer cells, compared to their free counterpart. Out of the two nanocarriers, liposomes presented with a much greater in cellular uptake compared to micelles for all designated time points. As a consequence, both liposomal and micellar p-ibuprofen nanocarriers were found to be more cytotoxic against cancer cells of all three cancer cell lines, compared to free p-ibuprofen. This anticancer effect was attributed to the greater concentrations of P-I within the cancer cells, since cytotoxicity testing did not report any significant toxicity values with regards to the delivery vehicles (Nie *et al*., 2011). In addition to that, encapsulated p-ibuprofen also reduced NSAID cytotoxicity in normal cells, suggesting the preferential targeting of tumor cells over healthy (Nie *et al*., 2011).

With regards to drug biodistribution, the paper mentions that intact p-ibuprofen could be readily detected in murine organs including the heart, lungs, liver, kidneys and intestine following intraperitoneal injection of liposomes. This proves the ability of liposomal p-ibuprofen to be successfully distributed in tissues around the body despite the short half-life of the drug compound (Nie *et al*., 2011). Although the

esterase activity in serum was high, a percentage of 40% intact p-ibuprofen was detected after the administration of liposomes, but this was not the case for non-encapsulated free drug. Similar findings were also observed in tissues, with the gastrointestinal organs having the highest levels of intact p-ibuprofen, and the lungs having the lowest. The major metabolite present in these tissues was ibuprofen, as a result of the limited phospho-ibuprofen bioavailability in the absence of protection of liposomal constructs from degradation (Nie *et al*., 2011). With respect to pharmacokinetics, the administration of free p-ibuprofen was associated with the detection of ibuprofen, but not intact p-ibuprofen, in whole murine blood 30 minutes post-injection. On the other hand, liposomal P-I was spotted at high levels in whole blood following the administration of liposomes, proving that liposomes offer protection against esterase hydrolysis *in vivo*. The levels of intact drug decreased rapidly and became undetectable 30 minutes after the administration of liposomes. Metabolites of p-ibuprofen were also found to be at higher levels in plasma after the administration of liposomes, compared to free drug (Nie *et al*., 2011). Last but not least, the anticancer activity of liposomal p-ibuprofen was also higher than that of its free counterpart. Liposome-encapsulated P-I reduced the volume of tumors by 51%, reaching statistical significance at day 5 of the treatment, whereas free p-ibuprofen reduced tumor volumes by 23% and this reduction was not deemed statistically significant. Interestingly enough, both free and encapsulated p-ibuprofen reduced tumor weights by 73% and 45%, respectively. The liposome-encapsulated compound, however, was more efficient than free P-I, and this increased efficacy can be attributed to the increased biodistribution and bioavailability of the former (Nie *et al*., 2011).

This study suggests the use of nanocarriers as a potential alternative to the use of free p-ibuprofen for the treatment of cancer. This alternative has been shown to cause a significant increase in the bioavailability and biodistribution of P-I, as well as a noteworthy decrease in tumor volumes, compared to free P-I. The application of this treatment within a clinical setting could potentially bear interesting results in human models regarding the use of nano-encapsulated p-ibuprofen (Nie *et al*., 2011). The use of nanocarriers for the encapsulation of other phospho-NSAIDs should also be considered as a future research topic. Phospho-NSAIDs have been shown to inhibit the growth of tumor cells much more efficiently, and therefore their

encapsulation using nanocarriers could further enhance their chemotherapeutic potential (Xie *et al*., 2011).

In another paper published in 2014, Marques and their team synthesized and characterized micelles from amphiphilic chitosan as potential nanocarriers for the targeted delivery of ibuprofen into breast cancer cells.

Marques *et al*. (2014) initially made use of an amino coupling chemistry in order to synthesize amphiphilic chitosan through the incorporation of leucine or 3α, 12α-Dihydroxy-5β-cholanate (deoxycholic acid (DOCA)) into the chitosan backbone. These amphiphilic chitosan derivatives where then characterized by proton nuclear magnetic resonance (NMR) spectroscopy, whereas the successful inclusion of leucine and DOCA was confirmed using Fourier-transform infrared spectroscopy (FTIR) (Marques *et al*., 2014). Energy-dispersive X-ray spectroscopy (EDX) was used to determine the different chemical composition of the synthesized chitosan-DOCA and chitosan-leucine amphiphilic polymers. The critical micelle concentration (CMC) of the chitosan polymers was assessed through the pyrene encapsulation method in fluorescence spectroscopy. Following determination of the polymer CMC, the self-assembly of the amphiphilic chitosan micelles was stimulated by stirring sonication (Marques *et al*., 2014). The encapsulation efficiency of ibuprofen by the chitosan micelles was calculated using an UV-vis spectrophotometer. Micelle concentrations ranging from 0.1 to 1mg/mL were used to assess different micelle encapsulation capacities. The size and zeta potential of the synthesized micellar constructs was calculated using the dynamic light scattering (DLS) technique, following the self-assembly of micelles and the encapsulation of ibuprofen. More specifically, the zeta potential of the amphiphilic micelles was determined by the Smoluchowski model, whereas the Cumulants/Correlogram analysis was used for the successful determination of the micelle size (Marques *et al*., 2014). Visualization of the micelle morphology was achieved through the scanning electron microscopy (SEM) technique. Breast cancer cells deriving from the MCF-7 cell line were used to test the cytotoxicity of both polymer and micellar constructs. Different concentrations of polymer with a range of 5-200μg/mL were used to incubate MCF-7 cells. Blank micelles were also used to incubate breast cancer cells as a control (Marques *et al*., 2014). The Resazurin assay was carried out to monitor the potential cytotoxicity of the

chitosan micelles. Shortly after the evaluation of the micelle biocompatibility, micellar constructs were fluorescently labeled with the cell-permeant fluorogenic dye rhodamine B isothiocyanate (RITC), and their uptake by tumor cells was characterized by confocal laser scanning microscopy (CLSM). The Resazurin assay was also useful in determining the anti-proliferative activity of the different micellar constructs. MCF-7 cells were incubated at 24, 48 and 72 hours with the Resazurin reagent, and the intensity of fluorescence generated by these breast cancer cells was used to determine cell viability (Marques *et al*., 2014). Finally, all results were statistically expressed and compared through ANOVA, with a p value of less than 0.05 (< 0.05) being taken into consideration as a value of statistical significance (Marques *et al*., 2014).

Results of this study indicate that the inclusion of both leucine and DOCA into the chitosan backbone was successful. The degree of substitution, however, was found to be higher for the chitosan-DOCA polymers, compared to the chitosan-leucine derivatives (Marques *et al*., 2014). Their findings also suggest that the self-assembly of micelles occurs at relatively low concentrations of polymer for both chitosan-DOCA and chitosan-leucine. In detail, the optimal polymer concentration for micellar self-assembly was found to be 0.101mg/mL for chitosan-DOCA and 0.065mg/mL for chitosan-leucine polymers. This study also demonstrated that self-assembly of chitosan-leucine micelles can occur at lower polymer concentrations than these reported by previous literature (0.090mg/mL) (Layek and Singh, 2013; Marques *et al*., 2014). Similarly, chitosan-DOCA polymers were also reported to self-assemble into micelles at lower concentrations than these reported elsewhere (Li *et al*., 2010; Marques *et al*., 2014). These lower CMC values are highly indicative of the increased stability of self-assembled nanocarriers in aqueous solutions, therefore supporting their great potential as delivery vectors for therapeutic agents (Marques *et al*., 2014). Chitosan-leucine amphiphilic micelles presented with high ibuprofen encapsulation efficiencies for all different concentrations of the polymer. Results were fairly similar for chitosan-DOCA polymers, with the highest ibuprofen encapsulation efficiency being achieved at a chitosan-DOCA concentration of 0.3mg/mL. Both types of polymeric micelles were spherical, with all DOCA-incorporating nanocarriers presenting with a positive

surface charge, and all leucine-incorporating nanocarriers having a negatively charged surface (Marques *et al*., 2014).

All cells in this study incubated with hydrophobic chitosan derivatives expressed high viability, even at maximum concentrations of polymer, therefore suggesting an increase in biocompatibility, compared to the administration of raw nonsteroidal anti-inflammatory drugs (Marques *et al*., 2014). Chitosan-DOCA micelles caused cell proliferation after 48 hours, compared to non-incubated cells. Chitosan-leucine micelles, on the other hand, initiated a slight tumor cell proliferation at both 24 and 48 hours after administration. Blank control micelles were found to have no effect on breast cancer cell viability, regardless of their chemical composition (Marques *et al*., 2014). Fluorescently labeled micelles were readily detected in the cytoplasm of breast cancer cells 4 hours following administration, indicating the successful uptake of both amphiphilic polymers. Most importantly, the administration of micelles loaded with ibuprofen resulted in a decrease in tumor cell viability for all amphiphilic formulations, compared to untreated breast cancer cells (Marques *et al*., 2014). The treatment of tumor cells with free ibuprofen reportedly had a greater anti-tumor effect at 24 hours than that observed in cells incubated with ibuprofen-loaded amphiphilic micelles. However, administration of free ibuprofen is reported to be associated with low drug bioavailability and non-specific tissue toxicity, as reported by previous literature (Prakash *et al*., 2012). Ibuprofen-loaded micelles consisting of chitosan-DOCA polymers had a strikingly higher anti-tumor effect, compared to their chitosan-leucine counterpart, especially at concentrations of 0.3 and 0.4 mg/mL (Marques *et al*., 2014). Surprisingly, the anti-proliferative activity of these formulations was very similar to that observed with free ibuprofen. Moreover, the findings of this study imply that the anti-proliferative activity of the chitosan-DOCA amphiphilic micelles is even comparable with that of standard chemotherapy drugs, such as doxorubicin. Both doxorubicin and chitosan-DOCA micelles are found to cause similar reductions in tumor cell viability, although this reduction is achieved at a later time frame for chitosan-DOCA micelles (Ke *et al*., 2013; Marques *et al*., 2014).

Overall, this study proved that self-assembling micelles containing DOCA or leucine moieties can be engineered in order to efficiently facilitate both the encapsulation and delivery of NSAIDs with poor water solubility. These polymers

can form micelles under mild conditions and still express the high biocompatibility of unmodified chitosan (Marques *et al*., 2014). Moreover, ibuprofen-loaded micelles were found to decrease viability in MCF-7 breast cancer cells, in a manner that is comparable to commonly used chemotherapeutics, such as doxorubicin (Marques *et al*., 2014). This finding highlights the potential of micelles to be used in modern oncology as novel nanocarriers, especially for the delivery of poor water soluble anti-tumor drugs, including NSAIDs.

4.0 Future Perspectives of Nano-NSAIDs in Modern Oncology

The use of NSAIDs in the prevention and treatment of cancer is a relatively new and promising field in modern medicine. Despite the fact that the involvement of nanotechnology in this field has yielded some interesting finding that suggest that nano-NSAIDs might have a more profound effect in the prevention and treatment of various types of tumors, there are still some steps that need to be taken, in order for the full potential of nano-NSAIDs in chemotherapy to be completely realized. Various studies have focused in the encapsulation of NSAIDs in nanocarriers, as well as in combining the chemotherapeutic activity of NSAIDs with other, nanoscale therapeutics.

4.1 Novel Nanocarriers: Liposomal Constructs

It is common knowledge among health professionals that most therapeutic anticancer drugs are administered either hypodermically or orally. Other methods of administration, however, have slowly started to gain ground throughout the years. As of now, transdermal delivery of drugs is the third commonest method of drug administration, following subcutaneous and oral delivery (Jiang *et al*., 2020). The utilization of liposomal constructs in transdermal delivery of drugs (TDD) has been thoroughly studied by scientists, mostly due to the high efficacy of liposomes, their native character, as well as other advantages associated with this route of administration (Elsayed *et al*., 2007). Moreover, studies have reported that the addition of penetration enhancers (PEs) has resulted in the extension of liposomes over the skin, which is the first step in order to accomplish a more systemic effect (Vázquez-González *et al*., 2014).

In light of this evidence, Vázquez-González *et al*. (2019) tested planar lipid bilayer derived from liposomes as potential carriers for transdermal drug delivery.

In order to achieve that, lipid monolayers were prepared, and their elasticity and thermodynamics were calculated. The initial objective for the formation of liposomes was to use the main components of the *stratum corneum*, the outermost layer of the epidermis, cholesterol (CHOL), ceramides (CER) and free fatty acids. However, due to the fact that free fatty acids have a tendency to form micellar structures when in solution, they were replaced by L-α phosphatidylcholine (PC), in order to achieve liposome formation, improved

performance and stability (Vázquez-González *et al*., 2019). Three different types of liposomes were formed using appropriate techniques: unloaded liposomes, liposomes loaded with the nonsteroidal anti-inflammatory drug ibuprofen, and liposomes loaded with hyaluronic acid. For both the ibuprofen- and hyaluronic acid-loaded liposomes, the penetration enhancer Tween ® 80 was added in order to ensure the partial destabilization of the lipid bilayer, thus promoting its modification into planar lipid structures upon skin contact (Vázquez-González *et al*., 2019). The encapsulation efficiency, particle size and ζ potential and fluorescence of the liposome constructs were measured following the addition of the penetration enhancer. A spectrofluorometer was used to obtain information on the bilayer fluidity-dependent fluorescence, utilizing the dipolar relaxation of Laurdan, a probe that undergoes a redshift when the membranes are in the fluid phase (Vázquez-González *et al*., 2019). Human skin was prepared for the assessment of planar lipid structures on the skin and their subsequent extension. Atomic force microscopy was used to obtain pictures noting the morphology and roughness of the skin. Lastly, results obtained through atomic force microscopy were expressed statistically (Vázquez-González *et al*., 2019).

For the binary system containing phosphatidylcholine and cholesterol, results of this study indicate that maximum liposomal stability can be achieved with a PC:CHOL ratio of 0.6:0.4 mol/mol. The subsequent, proportional addition of ceramides resulted in the formation of a more stable, ternary lipid monolayer, and the maximum stability for the ternary lipid system was achieved at a PC:CHOL:CER ratio of 0.36/0.24/0.24 mol/mol/mol (Vázquez-González *et al*., 2019). The ternary lipid system PC:CHOL:CER was found to have the lowest Gibbs energy of mixing values, therefore implying its suitability as a drug vector for topical administration (Vázquez-González *et al*., 2019).

The incorporation of ibuprofen and hyaluronic acid into these liposomal structures resulted in slight increases in the size of the total product. The polydispersity index (PDI) remained similar in all cases, and the zeta potential was reported to always give negative values. A further decrease of the zeta potential was evident following the addition of the penetration enhancer Tween ® 80, a result that was expected to be of importance with regards to the extension of liposomes over the skin (Vázquez-González *et al*., 2019). The presence of non-phase-separated

liposomes in the fluid phase was proven by Laurdan fluorescence. The results are indicative of the surface extension of liposomes due to increased fluidity. Images obtained through atomic force microscopy suggest that the incorporation of hyaluronic acid enhances the extension over human skin and promotes the formation of planar lipid structures upon skin contact, a result that was not so evident with the incorporation of ibuprofen. Supplementation of liposomes with the penetration enhancer did not enhance the extension of liposomes over the skin, although an enhancing effect was apparent on the permeation of liposomes through the stratum corneum (Vázquez-González *et al*., 2019). Although results were not very encouraging with regards to the incorporation of ibuprofen, further studies should be addressed. Based on the fact that this experimental procedure was performed on the microscale, it is important that many factors, including lipid concentration and drug concentration gradient, should be evaluated in order to be applicable to the nano scale. The unique properties of particles exploited on the nano scale should also be taken into account for the development of novel therapies against cancer (Vázquez-González *et al*., 2019).

Another study (Gai *et al*., 2018) focused on the development of a novel bioadhesive colloidal system consisting of cationic liposomal constructs for the ocular delivery of ibuprofen. Cationic liposomes appear to be a very promising candidate for the delivery of therapeutic agents to the oculi, mostly due to the fact that they can increase the retention time, permeation and bioavailability certain drugs, including the hydrophobic ibuprofen, and their inherent ability to form electrostatic bonds between the liposomal constructs and the negative charges of the ocular mucin layer (Cortesi *et al*., 2006).

Gai *et al*. (2018) prepared cationic liposomes of ibuprofen, ibuprofen-neutral liposomes, and a suspension of ibuprofen eye drops using soybean phosphatidylcholine, cholesterol, and octadecylamine. The encapsulation efficiency of cationic liposomes of ibuprofen was determined by the four following factors: soybean phospholipid/cholesterol ratio, drug/lipid ratio, the octadecylamine/soybean phospholipid ratio and the temperature of the water bath (Gai *et al*., 2018). The encapsulation efficiency obtained through the single-factor test mentioned above, was then used to evaluate the optimal cationic liposome preparation through an orthogonal test. Particle size distribution and the zeta

potential of particles were assessed using appropriate techniques (Gai *et al*., 2018). Gel permeation chromatography was carried out in order to test how effectively ibuprofen had been entrapped in the liposomal constructs, whereas transmission electron microscopy was used to observe their morphology. Cationic liposome stability was measured at 10, 20, and 30 days and the leakage rate of ibuprofen was calculated (Gai *et al*., 2018). In vivo studies included the determination of ibuprofen elimination kinetics through single photon emission computing tomography. Elimination kinetics were determined through encapsulation and detection of the metastable Technetium-99m instead of ibuprofen. Transcorneal permeation, the cumulative released quantity of ibuprofen and the hydration levels of the cornea were tested in vitro, using rabbit corneas (Gai *et al*., 2018). High-performance liquid chromatography was performed for determination of the total ibuprofen concentration of samples. Gai et al. utilized the microdialysis technique and high-performance liquid chromatography to study the pharmacokinetics of the samples. Results obtained during this study were expressed statistically using the Student's two one-sided t test. Statistically significant differences were considered at $p<0.05$ (Gai *et al*., 2018).

For this study, the ideal soybean phosphatidylcholine:cholesterol ratio for liposome preparation was found to be 6:1. This factor had the largest impact on the encapsulation of ibuprofen, and findings were consistent with the idea that soybean phospholipids take up most of the lipid bilayer, whereas the main role of cholesterol lies in influencing the membrane fluidity (Koklic, 2014; Gai *et al*., 2018). With regards to the drug/lipid ratio, it was apparent that an increasing concentration of lipid subsequently increased liposomal drug efficacy, but an increase of the drug:lipid ratio to 1:12 resulted in a slight decrease in encapsulation efficiency. For the octadecylamine:soybean phospholipid ratio, it was obvious that increasing concentrations of octadecylamine resulted in a decreased encapsulation efficiency of ibuprofen, whereas the ideal ratio was at 1:12. The ideal temperature of the waterbath was found to be 50^{o}C. Ibuprofen was entrapped in between the lipid bilayer of cationic liposomes (Gai *et al*., 2018). Results of this study indicate that liposomes presented with the highest stability when refrigerated for up to 30 days. Ibuprofen-loaded cationic liposomes presented with a favorable profile for both in vivo and in vitro studies. Not only did

they showcase a prolonged ocular residence time and a promoted cumulative release of the drug, but they also displayed an enhanced release rate and corneal transpermeability, compared to ibuprofen eye drops and neutral liposomes (Gai *et al*., 2018).

Overall, this study further supports the idea that liposomes can act as ideal vesicles for the delivery of NSAIDs for topical administration. Moreover, the need to effectively regulate the total concentrations of lipids in order to produce liposomes of the best quality is made apparent (Gai *et al*., 2018). Further studies should be conducted in order to assess the delivery of NSAIDs and other chemopreventive and chemotherapeutic agents to cancerous tissue, as the idea itself seems very promising to the field of both Nanomedicine and Modern Oncology.

4.2 Novel Nanocarriers: Hydrogels

In addition to liposomal constructs, the use of nanoparticle hydrogels for the delivery of NSAIDs is an emerging alternative that yields very promising results. In a recent article, Chen *et al*. (2019) evaluated the use of a thermosensitive nanoparticle nanogel for the prolonged co-delivery of ibuprofen and basic fibroblast growth factor (bFGF) for the treatment of early local peri-implantitis.

For these experiments, Chen and their scientific team (2019) prepared a thermosensitive poly(ε-caprolactone-co-1, 4, 8, trioxa [4.6]spiro-9-undecanone) (PECT) hydrogel of micellar nature and loaded it with both ibuprofen and bFGF. Unloaded PECT hydrogels were also prepared to serve as controls for this paper. Mathematical calculation of *in vitro* drug release from the PECT hydrogels was carried out for 6, 12 and 18 days, using high performance liquid chromatography for the detection of ibuprofen in the supernatant, and UV spectrophotometry for the assessment of bFGF release through simulation with BSA-fluorescein isothiocyanate conjugate (FITC). IBU and BSA-FITC concentrations were calculated using a standard curve (Chen *et al*., 2019). Primary human gingival fibroblasts (HGFs) were cultured in parallel with hydrogel preparation, in order to evaluate the potential cytotoxic effects of PECT hydrogels using cell counting kit-8 (CCK-8) colorimetric assays. PECT biocompatibility was tested five times pre group, using various PECT concentrations. Sample absorbance was measured at

450nm (Chen *et al*., 2019). CCK-8 assay was also performed for 1, 3, 5 and 7 days for the optimization of the concentration of bFGF that would efficiently promote HGF growth. Titanium discs were then seeded with HGFs for an HGF proliferation assay and cells were randomly cultured in triplicate under three different conditions: growth medium, bFGF, and ibuprofen/bFGF PECT hydrogels. Proliferation of HGFs with the ibuprofen/bFGF PECT hydrogels was evaluated CCK-8, whereas the long-term effects of PECT were assessed in triplicate using Transwell chambers (Chen *et al*., 2019). Adhesion of HGFs to the titanium discs was verified through fluorescent antibodies. Quantitative real-time PCR was carried out to evaluate the effects of drug loaded PECT hydrogels on the expression of the mammalian protein vinculin, and a reverse transcription reagent kit was used to convert RNA obtained through PCR into cDNA. The $2^{-\Delta\Delta Ct}$ method was used to calculate gene expression. Last but not least, the expression of the inflammatory cytokine prostaglandin E2 by HGFs was investigated using the enzyme linked immunosorbent assay (ELISA). Results of this study were statistically analyzed using ANOVA (Chen *et al*., 2019).

Findings of this study suggest that thermosensitive PECT hydrogels loaded with bFGF and ibuprofen can effectively promote the proliferation and adhesion of human gingival fibroblasts and suppress inflammatory responses. In addition to that, PECT hydrogels expressed a favorable sustained-release profile that was highly controllable and managed to maintain the release of both hydrophilic bFGF and hydrophobic ibuprofen (Chen *et al*., 2019). This finding could further expand to include more hydrophilic and hydrophobic drugs, therefore rendering hydrogels polyvalent carriers for the delivery of various therapeutic agents. This utilization of thermosensitive hydrogels for the delivery of NSAIDs could potentially prove to be of great importance in the field of oncology, since cancerous tissues have been found to express higher temperatures compared to their healthy counterpart. The comparatively lower pH values observed in tumors could also be used for the development of pH-sensitive nanocarriers for the delivery of NSAIDs and other chemotherapeutic drugs (Lawson and Chughtai, 1963; Wojtkowiak *et al*., 2011).

4.3 Novel Nanocarriers: Enhanced Delivery to Tumors

Steps can also be made in order to improve the delivery of nonsteroidal anti-inflammatory drugs to cancer tissue. Angelini *et al*. (2017) studied the effects of entrapping a β-cyclodextrin/ibuprofen inclusion complex to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) liposomal carriers. Cyclodextrins are commonly used for the generation of host-guest inclusion complexes, due to their ability to form frail interactions between molecules and to enhance the water solubility of lipophilic substances (Uekama *et al*., 1998; Rekharsky and Inoue, 1998).

Angelini and their team (2017) prepared a β-cyclodextrin/ibuprofen inclusion complex, whose formation was then spectrophotometrically determined using excitation and emission wavelengths of 262 and 288nm, respectively. Following formation of the β-CD/IBU complexes, multilammelar vesicles (MLVs) of POPC were prepared through the dehydration-rehydration method. POPC multilammelar vesicles were visualized using transmission electron microscopy (TEM) technique, followed by subsequent measurement of their kinetics and determination of their multilammelar microviscosity (Angelini *et al*., 2017). A population of large unilammelar vesicles (LUVs) was also generated through extrusion. The suspension containing those POPC LUVs was prepared by MLVs in both the presence and absence of β-cyclodextrin. Kinetics and the microviscosity of LUVs were also assessed during the experiments (Angelini *et al*., 2017).

The presence of β-cyclodextrin is responsible for enhancing the solubility of lipophilic drugs in aqueous solutions. Lipophilic drugs, however, have to be of appropriate size in order to be included in the macrocyclic structure of cyclodextrins (Angelini *et al*., 2017). The stability and microviscosity of POPC MLVs containing the β-cyclodextrin/ibuprofen inclusion complexed was found by scientists to be dependent on other factors, including the liposomal composition of generated liposomes, the macrocycle molecules and the drugs used for preparation of the inclusion complexes. The presence of free β-cyclodextrin resulted in a loss of stability and a reduction of the microviscosity of POPC multilammelar vesicles, whereas the inclusion of β-cyclodextrin/ibuprofen complexes bore contrasting results, mostly due to the free drug gradually transferred from the inclusion complex penetrating the POPC membrane bilayer

(Angelini *et al*., 2017). Low concentrations of ibuprofen were found to contradict the effects of β-cyclodextrin, therefore stabilizing the membrane bilayer. A synergistic effect of ibuprofen and β-cyclodextrin was observed at high local concentrations (Angelini *et al*., 2017). Their results represent a good example of how the entrapment of β-CD/drug inclusion complexes into the aqueous compartments of MLVs can be used to effectively control drug release due to membrane bilayer disintegration (Angelini *et al*., 2017). A possible role of ibuprofen and other anti-inflammatory drugs in this setting is also implied, with potential applications in the field of modern oncology. The novel properties attributed to the utilization of particles in the nano scale might also be of great importance in improving the stability, viscosity and drug release of liposomal constructs (Angelini *et al*., 2017).

4.4 Novel Nanocarriers: Nanocarriers Exerting Novel Properties

The future of the incorporation of nonsteroidal anti-inflammatory drugs in modern oncology should also involve the synthesis of nanocarriers with various properties and characteristics, in order to synergistically enhance the chemopreventive and chemotherapeutic role of NSAIDs. A research paper by Hasegawa *et al*. (2016) discusses the synthesis of nitric oxide-donating furoxan-bearing micelles for the release of nitric oxide in response to cysteine, as well as the additional amplification of the anti-proliferative effect of ibuprofen in human colon cancer. As mentioned above, it has been suggested by previous reports that nitric oxide can cause the sensitization of tumor cells to various chemotherapy drugs and NSAIDs (Rigas and Kashfi, 2004; Zhao *et al*., 2012).

Hasegawa and their team (2016) polymerized N-(2-hydroxymethyl)acrylamide (HEA), using 2,2'-Azobis(isobutyronitrile) (AIBN) as the initiator and 2-(((dodecylthio)carbonthioyl)thio)-2-methylpropanoic acid as the chain transfer agent. Degasification of the solution was performed using argon in five freeze-thaw circles, followed by their subsequent heating for 24 hours. Liquid nitrogen was used to stop the polymerization process (Hasegawa *et al*., 2016). Following the synthesis of the HEA polymer (PHEA), similar protocols were followed for the polymerization of 4-Acryloylmorpholine (AM), the synthesis of a PAM-PHEA diblock copolymer, the removal of the chain transfer agent through radical-induced

reduction using tris(trimethylsilyl)silane, and the synthesis of fluorescently labeled and non-labeled furoxan-bearing block copolymers. The formation of micelles from the furoxan-bearing block copolymers was achieved through their dissolution in dimethylformamide (DMF), followed by the subsequent overnight dialysis of the solution in water. The contact angle versus concentration curve was then used for the calculation of the CMC of micellar solutions at different concentrations (Hasegawa *et al*., 2016). NO_2- concentration was calculated using the Griess assay. For the Griess test, samples were reacted with sulfanilamide and hydrochloric acid prior to the addition of N-(1-naphthyl)-ethylenediamine dihydrochloride. The absorbance was measured at 550nm after 5 minutes and the concentrations of NO_{2-} were calculated from standard NO_{2-} curves in either cysteine or in cysteine in Triton X-100 in phosphate-buffered saline containing diethylene triamine pentaacetic acid (DTPA) (Hasegawa *et al*., 2016). In this study, a furoxan derivative was also prepared and used as a model compound. The chemical structure of the furoxan derivative was similar to that of the polymer-bound furoxan, with the difference that the aliphatic polymer backbone of the latter was replaced by a methyl group. The model compound should therefore have the same reactivity as the polymer in the presence of cysteine. The Griess assay was later used in a similar manner in order to evaluate the release of nitric oxide from the micelles and the model compound in the presence of cysteine in DTPA-containing phosphate-buffered saline, Triton X-100, and following incubation in phosphate-buffered saline (Hasegawa *et al*., 2016). The stability of both furoxan-bearing micelles and the model compound was evaluated through dilution with an equal volume of phosphate-buffered saline containing DTPA. Samples were kept for 0, 1, 3 and 7 days prior to measuring with ultraviolet-visible spectroscopy. Human colon cancer cells deriving from the HT-29 cell line were cultured and cancer cells obtained from cell cultures were then subjected to trypsinization and passaging upon reaching a confluence rate of 70-80%. Hasegawa and their team (2016) also tried to assess whether the nitric oxide-releasing furoxan-bearing micelles were able to exert a synergistic effect to the anti-proliferative activity of ibuprofen. Human colon cancer cells were incubated with micelles without ibuprofen, ibuprofen and mixture of micelles, model compound with and without ibuprofen, and ibuprofen in water/dimethylsulfoxide (DMSO). Cells well treated with medium alone as a control for this experiment. Cells were cultured for two

days, and then the medium was removed and MTT assays were performed. After 2 hours, formazan crystals formed during the MTT assay were dissolved in sodium dodecyl sulfate (SDS) and hydrochloric acid and the absorbance was measured at 570nm (Hasegawa *et al*., 2016). All results obtained were expressed statistically, using the Student's t-test. Finally, the uptake of fluorescently labeled micelles in human colon cancer cells was visualized by confocal laser scanning fluorescence microscopy (Hasegawa *et al*., 2016).

The synthesis of an amphiphilic block copolymer including a hydrophobic furoxan-bearing block and a hydrophilic poly(N-acryloylmorpholine) block was successful in this experiment. Subsequent preparation of the nitric oxide-releasing furoxan-bearing micelles was achieved through self-assembly in water (Hasegawa *et al*., 2016). The prepared micellar constructs were reported to have a diameter of approximately 50nm, which is in agreement with the size range of 10-100nm that is typical for polymer spherical micelles of the core-shell type (eds. Kobayashi and Müllen, 2004; Hasegawa *et al*., 2016). Transmission electron microscopy and atomic force microscopy techniques were used to confirm the spherical morphology of the synthesized micelles. Observation of the micellar constructs using transmission electron microscopy, however, showed nanoparticles with a diameter of approximately 15nm. This finding is due to the fact that the shell of micellar constructs is not visible by transmission electron microscopy using the negative staining method (Hasegawa *et al*., 2016). Moreover, this study reported a CMC of 0.8×10^{-6}M, a finding that supports the idea that furoxan-bearing block copolymers are hydrophobic enough to self-assemble in order to form micelles. Previous studies suggest that the presence of cysteine and other thiol-containing compounds can promote the release of nitric oxide from furoxans (eds. Wang *et al*., 2005). This study further confirmed previous reports, as it has shown that the presence of cysteine induces the release of nitric oxide from micelles in a concentration-dependent manner (Hasegawa *et al*., 2016). Comparing the release of nitric oxide from the micelles and the model compound showed a noteworthy difference, with the latter showcasing a much higher amount of nitric oxide release after 3 days. This finding is believed to be due to the limited interaction between the furoxan located within the hydrophobic micellar core and cysteine. This hypothesis was further supported by the fact that the addition of Triton X-100

resulted in an increased release of nitric oxide from micelles compared to the micelles alone, therefore proving that the release of nitric oxide is critically affected by the micellar structure. The micelles, however, were found to be more stable in this study, whereas the furoxan-derived model compound presented with notable spectral changes and had a reduced release of nitric oxide over the course of 1 week (Hasegawa *et al*., 2016). With regards to the synergistic relationship between NSAIDs and the micellar constructs, it was shown that neither of them could cause a significant reduction in human colon cancer cell viability. The combined administration of ibuprofen and micelles, however, yielded strong anti-tumor results. More importantly, these furoxan moiety-bearing micelles showcased a successful internalization by HT-29 cells, even after 2 hours (Hasegawa *et al*., 2016).

This study suggests that the involvement of nanocarriers in modern oncology may also affect the chemotherapeutic and chemopreventive activity of NSAIDs in an indirect manner, especially through the exertion of a synergistic effect (Hasegawa *et al*., 2016). It is possible that, in this setting, the replacement of ibuprofen with other NSAIDs that carry a nitric oxide-releasing moiety could further enhance the anti-proliferative and pro-apoptotic effects in different tumor types and yield surprising results. Overall, NSAIDs carrying a nitric oxide-releasing moiety have been reported to have a stronger chemotherapeutic activity in various cancer cells compared to uncoupled NSAIDs (Williams *et al*., 2001). Nitric oxide-releasing NSAIDs are a relatively novel class of medications that is generated through the addition of nitrosothiol or nitroxybutyl to the parent NSAID molecule. Examples of these novel, nitric oxide-bearing NSAIDs include NO-flurbiprofen, NO-aspirin and NO-naproxen (Fiorucci *et al*., 2001).

The known anticancer activity of NSAIDs can also be exploited, even in cases where nonsteroidal anti-inflammatory drugs are not the primary therapeutic agent for cancer treatment. In a paper from 2016, Zhao and colleagues attempted to synthesize nanomicelles of PEG-derivatized ibuprofen for the encapsulation of the chemotherapy drug paclitaxel. These conjugates were tested on four different cancer cell lines in order to evaluate the presence of a synergistic effect between ibuprofen and paclitaxel. The four cancer cell lines used in this study comprise the

metastatic breast cancer cell line 4T1.2, the human breast cancer cell line MCF-7, and the androgen-dependent human prostate cancer cell lines DU145 and PC-3.

For this experiment, Zhao *et al*. (2016) synthesized PEG-derivatized ibuprofen, PEG_{2K}-FIbu. At first, Zhao and colleagues mixed poly-(ethylene glycol) methyl ether (MeO-PEG-OH) with N-alpha-(9-Fluorenylmethyloxycarbonyl)-N-epsilon-t-butyloxycarbonyl-L-lysine (Fmoc-Lys(Boc)-OH) and N,N'-dicyclohexylcarbodiimide (DCC) in dichloromethane with the addition of 4-dimethylaminopyridine (DMAP). Following stirring for 48 hours, filtering and precipitation of the mixtures, Zhao *et al*. (2016) washed the mixtures twice with cold ethanol. Trifluoroacetic acid (TFA) in dichloromethane for the removal of Boc groups. PEG derivatives were later precipitated and washed twice using cold ether. DCC and DMAP were used for the coupling of ibuprofen to the exposed lysine amino acids. Purification by cold ethanol and ether precipitation were performed on the PEG-derivatized ibuprofen (Zhao *et al*., 2016). Thin-film hydration method was carried out for the preparation of free micelles and micelles loaded with the chemotherapy drug paclitaxel. The size of both paclitaxel-free and paclitaxel loaded micelles was measured using the dynamic light scattering technique, with the concentration of paclitaxel being kept stable at 1mg/mL (Zhao *et al*., 2016). Observation of the micellar morphology was achieved through transmission electron microscopy. 1% uranyl acetate was the dye of choice for this method. Moreover, the CMC of PEG-derivatized ibuprofen micelles was measured, with pyrene being used as a fluorescent probe. Pyrene was mixed with various concentrations of the micellar constructs, and the fluorescence intensity for each sample was measured at an excitation wavelength of 334nm and an emission wavelength of 390nm (Zhao *et al*., 2016).

Assessment of the loading efficiency of paclitaxel was done by high performance liquid chromatography, followed by ultraviolet light detection at a wavelength of 227nm. The drug loading capacity (DLC) of the micelles was calculated by dividing the weight of loaded paclitaxel with the total weight of the micellar construct, and then multiplying the result by 100. As for the drug loading efficiency (DLE) of the micelles, it was calculated by dividing the weight of the loaded drug with the weight of the input drug, and multiplying the quotient by 100 (Zhao *et al*., 2016). The team also studied the micellar release of paclitaxel in vitro. To achieve that, they placed 2mL of the drug-loaded micelles in dialysis bags containing Dulbecco's phosphate

buffered saline (DPBS) and 0.5% w/v Tween 80. Raw paclitaxel was also diluted in DPBS and used as a control. HPLC was used to measure the total concentrations of paclitaxel remaining in the dialysis bag at designated time points. Triplicate samples were used to obtain the values that were reported in this experiment (Zhao *et al*., 2016). Cell cultures of the four cell lines used in this paper were obtained using Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were seeded in wells and incubated for 24 hours prior to treatment with various concentrations of paclitaxel-loaded and unloaded micelles, as well as with raw paclitaxel. Cells were further incubated for 72 hours prior to being tested using the MTT assay. Untreated cancer cells were used as a control. The absorbance was measured at a wavelength of 550nm. Cell viability and the cytotoxicity of PEG-derivatized ibuprofen were calculated, with the latter also being compared to the cytotoxicity of free ibuprofen. Zhao and their team (2016) also assessed the distribution of the PEG-derivatized ibuprofen micelles to tissues. For that purpose, the team used female BALB/c bearing invasive breast cancer of the 4T1.2 cell line. Free paclitaxel and paclitaxel-loaded micelles were injected via tail vein injection when the volume of the tumors reached a range between 400 and 600mm^3. The dose of paclitaxel used in this experiment was 10mg/kg of body weight for both free and micelle-incorporated paclitaxel. Sacrifice of breast cancer-bearing mice was performed 24 hours after the injection, followed by the subsequent excision of major organs and solid tumors. Determination of paclitaxel levels was done using HPLC (Zhao *et al*., 2016). The therapeutic efficacy of paclitaxel-loaded carrier micelles was evaluated a syngeneic murine model of invasive breast cancer of the 4T1.2 cell line. 4T1.2 cells were inoculated to obtain a final tumor volume of approximately 50mm3. Cells were then divided into four different groups and treated with free paclitaxel, unloaded micelles, paclitaxel-loaded micelles, and phosphate buffered saline as a control. Administration of the compounds was done on days 1, 3, 5, 8, and 11. A digital caliper was used to measure the volume of the tumors. If and when the tumors reached a total volume of roughly 2000mm3, the mice were sacrificed. The rates of survival were expressed using Kaplan-Meier curves, whereas the body weight of mice was constantly recorded during the experiments to evaluate potential toxicity (Zhao *et al*., 2016). Biochemical staining of tissues was performed shortly after the in vivo therapeutic

study using hematoxylin and eosin (H&E) stain for visualization under a microscope. Results of this study were statistically analyzed using Student's t test and one-way ANOVA, followed by Newman-Keuls test for p values of less than 0.05. In all statistical tests performed, a p value of less than 0.05 was considered to be of statistical significance (Zhao *et al*., 2016).

Zhao and colleagues (2016) reported that both paclitaxel and ibuprofen had an inhibitory effect on 4T1.2 breast cancer cells when administered singly. However, co-administration of the two agents had a combinatory effect on cancer cell inhibition. This anticancer effect was greater for the combination of the two agents than for each one of them used separately (Zhao *et al*., 2016). The use of a combination index was also taken into account to further evaluate the combination effect of paclitaxel and ibuprofen. A combination index (CI) of less than 1 suggests the presence of a synergistic effect; a combination index of greater than 1 suggests the presence of an additive effect; and a combination index of 1 indicates the absence of any effect. The CI for the two agents used in this study was equal to 0.63, confirming the presence of a synergistic effect between paclitaxel and ibuprofen. This finding prompted the synthesis of micellar constructs with an incorporated ibuprofen motif for the delivery of paclitaxel to cancer tissues (Zhao *et al*., 2016).

The findings of this study suggest that PEG-derived ibuprofen could readily self-assemble to form micelles in an aqueous solution. The formed micelles were of small size of approximately 12nm. Results obtained through transmission electron microscopy and dynamic light scattering confirmed the presence of small particles of spherical size in a homogeneous manner of distribution (Zhao *et al*., 2016). The CMC value obtained for unloaded micelles was equal to 0.197μM. An interesting finding of this study was the increase observed in the size of micelles following the loading of the chemotherapy drug paclitaxel. Loaded particles were still within the nano range, as reported by both transmission electron microscopy and dynamic light scattering (Zhao *et al*., 2016). A molar ratio of paclitaxel to ibuprofen-incorporating micelles as low as 0.5:1 was deemed to be sufficient and achieve a relatively high paclitaxel loading capacity of approximately 67%, as well as a stability of 12 hours at room temperature. Further increases of that ratio were

found to enhance both the colloidal stability and the drug loading efficiency of the micelles (Zhao *et al*., 2016).

The *in vitro* drug release study showed a slower rate of paclitaxel release for the PEG-derivatized ibuprofen micelles, compared to the release rate from its raw Taxol formulation. The release rates were found to be 38.62% and 55.21%, respectively. Moreover, the T1/2 values regarding the release of paclitaxel from Taxol were 18.8 hours, whereas only 44% of paclitaxel had been released from the micelles even after 48 hours (Zhao *et al*., 2016). With regard to cancer cell cytotoxicity, the use of Taxol caused a concentration-dependent inhibition on cancer cell growth in all four cancer cell lines tested. The use of paclitaxel-loaded micelles showcased a similar anticancer effect for this experiment. Administration of the unloaded nanocarrier did not yield any significant chemotherapeutic effects, but this was speculated to be due to the low concentrations used in this experiment. Micelles loaded with paclitaxel had a 2-fold increased drug uptake in tumor tissues, and a reduced drug accumulation in a few normal organs including the lungs and liver, compared to Taxol. Therefore, this finding suggests an obvious increase in effectiveness and a reduction in normal cell cytotoxicity for the micellar constructs (Zhao *et al*., 2016). *In vivo* therapeutic studies using 4T1.2 breast cancer mice also displayed some interesting results. The use of the Taxol formulation resulted in a moderate inhibition of cancer cell growth at a dose of 10mg of paclitaxel per kg of body weight (mg/kg). However, the use of paclitaxel-loaded micelles exerted a significantly greater anticancer effect at the same dose (Zhao *et al*., 2016). Moreover, the use of unloaded ibuprofen micelles also had an inhibitory effect on tumor cell growth, despite it being less effective compared to Taxol. All treatment groups expressed no significant reductions in body weight, when compared to the control group. Murine subjects treated with the Taxol formulation and paclitaxel-loaded micelles also showed longer median times of survival of 23 and 26 days, respectively, compared to a median survival time of 21 days in mice treated with phosphate buffered saline (Zhao *et al*., 2016). Finally, paclitaxel-loaded nanoparticles had a more profound effect on tumor cells stained with H&E dye, compared to Taxol. More specifically, micelle administration resulted in a greater shrinkage of the cancer cell nuclei and a further decreased tumor density. Tumor treated with phosphate buffered saline as a control

presented with large nuclei due to their high rate of proliferation (Zhao *et al*., 2016).

In conclusion, this study by Zhao and their team focused on the synthesis of micelles carrying a built-in, hydrophobic ibuprofen motif for the delivery of the chemotherapy agent paclitaxel. The release of paclitaxel from the PEG-derivatized ibuprofen micelles was significantly slower compared to its release from its standard Taxol formulation. More importantly, the administration of micelles showcased a stronger and more profound anticancer effect *in vivo*, although the two therapeutic agents demonstrated similar amounts of cytotoxicity *in vitro* (Zhao *et al*., 2016). The incorporation of ibuprofen in the micellar constructs had a synergistic effect on the anticancer activity of paclitaxel. It is possible that the incorporation of a higher number of ibuprofen moieties could further amplify the chemotherapeutic and chemopreventive effects of paclitaxel. Zhao and colleagues (2016) also proposed the incorporation of a tumor microenvironment-responsive linkage between the carrier backbone and ibuprofen, aiming to enhance the synergy between the two drugs. Last but not least, it would be interesting to test whether administration of this nanoproduct would bear similar results in clinical trials.

5.0 Conclusion

The last decades have seen a noteworthy increase in the number of studies and papers focusing on the use of nonsteroidal anti-inflammatory drugs, such as aspirin and ibuprofen, for the treatment and prevention of cancer in various tissues and organs of the human body. Results of these studies have for the most part been encouraging, therefore building a strong case regarding the huge potential of these commonplace therapeutic agents in the field of modern oncology. Unfortunately, the adverse side effects associated with the frequent use of NSAIDs still remain, therefore limiting their widespread application in chemoprevention and chemotherapy. Such side effects include gastrointestinal bleeding and gastric ulceration, and a wide range of cardiovascular problems. The rapid increase of nanotechnology could not leave the field of modern oncology untouched, with a particular interest in the use of NSAIDs for the treatment of cancer. This review was written in hopes of providing insight into the administration of various, commonplace NSAIDs for the treatment of cancer, analyzing published papers incorporating the use of nanotechnology in NSAID-based chemotherapy, proposing future courses of action whilst underlining their therapeutic potentian, and hopefully inspiring future research on nanoscale-based NSAID chemotherapy.

References

Anderson, D., Najafzadeh, M., Gopalan, R., Ghaderi, N., Scally, A. J., Britland, S. T., Jacobs, B. K., Reynolds, P. D., Davies, J., Wright, A. L., Al-Ghazal, S., Sharpe, D., and Denyer, M. C., (2014), 'Sensitivity and specificity of the empirical lymphocyte genome sensitivity (LGS) assay: implications for improving cancer diagnostics', *The FASEB Journal*, 28(10), 4563 – 4570.

Angelini, G., Campestre, C., Boncompagni, S., and Gasbarri, C., (2017), 'Liposomes entrapping β-cyclodextrin/ibuprofen inclusion complex: Role of the host and the guest on the bilayer integrity and microviscosity', *Chemistry and Physics of Lipids*, 209, 61 – 65.

Anon., (n. d.), '*NSAID Selection Tool – NSAID Classification*' [online], available at: https://globalrph.com/medcalcs/nsaid-selection-tool-nsaid-classification/, [Accessed 28 July, 2020].

Anon., (2007), '*NIH Curriculum Supplement Series: Understanding Cancer*' [online], Bethesda (MD): National Institutes of Health, available at: https://www.ncbi.nlm.nih.gov/books/NBK20362/, [Accessed 2 August, 2020]

Anon., (2017), '*LiverTox: Clinical and Research Information on Drug-Induced Liver Injury – Aspirin*' [online], Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, available at: https://www.ncbi.nlm.nih.gov/books/NBK548900/, [Accessed 1 August, 2020]

Anon., (2017), '*LiverTox: Clinical and Research Information on Drug-Induced Liver Injury – Celecoxib*' [online], Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, available at: https://www.ncbi.nlm.nih.gov/books/NBK548579/, [Accessed 1 August, 2020].

Anon., (2020), '*LiverTox: Clinical and Research Information on Drug-Induced Liver Injury – Ibuprofen*' [online], Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, available at: https://www.ncbi.nlm.nih.gov/books/NBK547845/, [Accessed 1 August, 2020].

Anon., (2020), '*LiverTox: Clinical and Research Information on Drug-Induced Liver Injury – Naproxen*' [online], Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, available at: https://www.ncbi.nlm.nih.gov/books/NBK548159/, [Accessed 1 August, 2020].

Arnold, J., Furtmüller, P. G., and Obinger, C., (2003), 'Redox properties of myeloperoxidase', *Redox Report*, 8(4), 179 – 186.

Ashok, V., Dash C., Rohan, T. E., Sprafka, J. M., and Terry, P. D., (2011), 'Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk', *The Breast*, 20(1), 66 – 70.

Benet, L. Z., (2013), 'The Role of BCS (Biopharmaceutics Classification System) and BDDCS (Biopharmaceutics Drug Disposition Classification System) in Drug Development', *Journal of Pharmaceutical Sciences*, 102(1), 34 – 42.

Boelsterli, U. A., Zimmerman, H. J., and Kretz-Rommel, A., (1995), 'Idiosyncratic liver toxicity of nonsteroidal antiinflammatory drugs: molecular mechanisms and pathology', *Critical Reviews in Toxicology*, 25(3), 207 – 235.

Bonina, F. P., Puglia, C., Barbuzzi, T., de Caprariis, P., Palagiano, F., Rimoli, M. G., and Saija, A., (2001), 'In vitro and in vivo evaluation of polyoxyethylene esters as dermal prodrugs of ketoprofen, naproxen and diclofenac', *European Journal of Pharmaceutical Sciences*, 14(2), 123 – 134.

Brasky, T. M., Bonner, M. R., Moysich, K. B., Ochs-Balcom, H. M., Marian, C., Ambrosone, C. B., Nie, J., Tao, M. H., Edge, S. B., Trevisan, M., Shields, P. G., and Freudenheim, J. L., (2011), 'Genetic variants in COX-2, non-steroidal anti-inflammatory drugs, and breast cancer risk: The Western New York Exposures and Breast Cancer (WEB) Study', *Breast Cancer Research and Treatment*, 126(1), 157 – 165.

Breder, C. D., DeWitt, D., and Kraig, R. P., (1995), 'Characterization of inducible cyclooxygenase in rat brain', *Journal of Comparative Neurology*, 355(2), 296 – 315.

Breder, C. D., and Saper, C. B., (1996), 'Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide', *Brain Research*, 713(1-2), 64 – 69.

Burn, J., Gerdes, A. M., Macrae, F., Mecklin, J. P., Moeslein, G., Olschwang, S., Eccles, D., Evans, D. G., Maher, E. R., Bertario, L., Bisgaard, M. L., Dunlop, M. G., Ho, J. W. C., Hodgson, S. V., Lindblom, A., Lubinski, J., Morrison, P. J., Murday, V., Ramesar, R., Side, L., Scott, R. J., Thomas, H. J. W., Vase, H. F., Barker, G., Crawford, G., Elliott, F., Movahedi, M., Pylvanainen, K., Wijnen, J. T., Fodde, R., Lynch, H. T., Mathers, J. C., and Bishop, D. T., on behalf of the CAPP2 Investigators, (2011), 'Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomized controlled trial', *The Lancet*, 378(9809), 2081 – 2087.

Cabarkapa, S., Perera, M., McGrath, S., and Lawrentschuk, N., (2016), 'Prostate cancer screening with prostate-specific antigen: A guide to the guidelines', *Prostate International*, 4(4), 125 – 129.

Cao, C., Matsumura, K., Yamagata, K., and Watanabe, Y., (1995), 'Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain: Its possible role in the febrile response', *Brain Research*, 697(1-2), 187 – 196.

Cao, C., Matsumura, K., Yamagata, K., and Watanabe, Y., (1996), 'Endothelial cells of the brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1b: A possible site of prostaglandin synthesis responsible for fever', *Brain Research*, 733(2), 263 – 272.

Chakraborty, I., Das, S. K., Wang, J., and Dey, S. K., (1996), 'Developmental expression of the cyclo-oxygenase-1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids', *Journal of Molecular Endocrinology*, 16(2), 107 – 122.

Chan, A. T., and Detering, E., (2013), 'An emerging role for anti-inflammatory agents for chemoprevention', *Recent Results in Cancer Research*, 191, 1 – 5.

Chandrasekaran, S., Al-Ghananeem, A. M., Riggs, R. M., and Crooks, P. A., (2006), 'Synthesis and stability of two indomethacin prodrugs', *Bioorganic and Medicinal Chemistry Letters*, 16(7), 1874 – 1879.

Chen, W., Zhi, M., Feng, Z., Gao, P., Yuan, Y., Zhang, C., Wang, Y., and Dong, A., (2019), 'Sustained co-delivery of ibuprofen and basic fibroblast growth factor by thermosensitive nanoparticle hydrogel as early local treatment of peri-implantitis', *International Journal of Nanomedicine*, 14, 1347 – 1358.

Christakos, S., Ajibade, D. V., Dhawan, P., Fechner, A. J., and Mady, L. J., (2010), 'Vitamin D: Metabolism', *Endocrinology and Metabolism Clinics of North America*, 39(2), 243 – 253.

Chuang, H. C., Kardosh, A., Gaffney, K. J., Petasis, N. A., and Schönthal, A. H., (2008), 'COX-2 inhibition is neither necessary nor sufficient for celecoxib to suppress tumor cell proliferation and focus formation in vitro', *Molecular Cancer*, 7:38.

Coghill, A. E., Phipps, A. I., Bavry, A. A., Wactawski-Wende, J., Lane, D. S., LaCroix, A., and Newcomb, P. A., (2012), 'The Association Between NSAID Use and Colorectal Cancer Mortality: Results From the Women's Health Initiative', *Cancer Epidemiology, Biomarkers & Prevention*, 21(11), 1966 – 1973.

Cook, N. R., Lee, I. M., Zhang, S. M., Moorthy, M. V., and Buring, J. E., (2013), 'Alternate-Day Low-Dose Aspirin and Cancer Risk: Long-term Observational Follow-up of a Randomized Trial', *Annals of Internal Medicine*, 159(2), 77 – 85.

Correia, I., Arantes-Rodrigues, R., Pinto-Leite, R., and Gaivão, I., (2014), 'Effects of naproxen on cell proliferation and genotoxicity in MG-63 osteosarcoma cell line', *Journal of Toxicology & Environmental Health*, 77(14-16), 916 – 923.

Cortesi, R., Argnani, R., Esposito, E., Dalpiaz, A., Scatturin, A., Bortolotti, F., Lufino, M., Guerrini, R., Cavicchioni, G., Incorvaia, C., Menegatti, E., and Manservigi, R., 'Cationic liposomes as potential carriers for ocular administration of peptides with anti-herpetic activity', *International Journal of Pharmaceutics*, 317(1), 90 – 100.

Cronin-Fenton, D. P., Pedersen, L., Lash, T. L., Friis, S., Baron, J. A., and Sorensen, H. T., (2010), 'Prescriptions for selective cyclooxygenase-2 inhibitors, non-selective nonsteroidal anti-inflammatory drugs, and risk of breast cancer in a population-based case-control study', *Breast Cancer Research*, 12(2):R15.

Cuzick, J., Otto, F., Baron, J. A., Brown, P. H., Burn, J., Greenwald, P., Jankowski, J., La Vecchia, C., Meyskens, F., and Senn, H. J., (2007), 'Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement', *The Lancet Oncology*, 10(5), 501 – 507.

Dandah, O., Najafzadeh, M., Isreb, M., Linforth, R., Tait, C., Baumgartner, A., and Anderson, D., (2018), 'Aspirin and Ibuprofen, in bulk and nanoforms: Effects on DNA Damage in peripheral lymphocytes from breast cancer patients and healthy individuals', *Mutation Research, Genetic Toxicology and Environmental Mutagenesis*, 826, 41 – 46.

Dannhardt, G., and Kiefer, W., (2001), 'Cyclooxygenase inhibitors – current status and future prospects', *European Journal of Medicinal Chemistry*, 36(2), 109 – 126.\

Eberhart, C. E., Coffey, R. J., Radhika, A., Giardiello, F. M., Ferrenbach, S., and Dubois, R. N., (1994), 'Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas', *Gastroenterology*, 107(4), 1183 – 1188.

Elsayed, M. M., Abdallah, O. Y., Naggar, V. F., and Khalafallah, N. M., (2007), 'Lipid Vesicles for Skin Delivery of Drugs: Reviewing Three Decades of Research', *International Journal of Pharmaceutics*, 332(1-2), 1 – 16.

Farokhzad, O. C., and Langer, R., (2009), 'Impact of nanotechnology on drug delivery', *ACS Nano*, 3(1), 16 – 20.

Fiorucci, S., Antonelli, E., Burgaud, J. L., and Morelli, A., (2001), 'Nitric oxide-releasing NSAIDs: a review of their current status', *Drug Safety*, 24(11), 801 – 811.

Furtmüller, P. G., Burner, U., Jantschko, W., Regelsberger, G., and Obinger, C., (2000), 'Two-electron reduction and one-electron oxidation of organic hydroperoxides by human myeloperoxidase', *FEBS Letters*, 484(2), 139 – 143.

Gai, X., Cheng, L., Li, T., Liu, D., Wang, Y., Wang, T., Pan, W., and Yang, X., (2018), '*In vitro* and *in vivo* Studies on a Novel Bioadhesive Colloidal System: Cationic Liposomes of Ibuprofen', *AAPS PharmSciTech*, 19(2), 700 – 709.

Garavito, R. M., Malkowski, M. G., and DeWitt, D. L., (2002), 'The structures of prostaglandin endoperoxide H synthases-1 and -2', *Prostaglandins & Other Lipid Mediators*, 68 – 69, 129 – 152.

Gibb, W., and Sun, M., (1996), 'Localization of prostaglandin H synthase type 2 protein and mRNA in term human fetal membranes and decidua', *The Journal of Endocrinology*, 150(3), 497 – 503.

Gurpinar, E., Grizzle, W. E., and Piazza, G. A., (2013), 'COX-Independent Mechanisms of Cancer Chemoprevention by Anti-Inflammatory Drugs', *Frontiers in Oncology*, 3:181, eCollection 2013, https://doi.org/10.3389/fonc.2013.00181.

Han, A. J., Kim, J. I., Ongusaha, P. P., Hwang, D. H., Ballou, L. R., Mahale, A., Aaronson, S. A., and Lee, S. W., (2002), 'p53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis', *The EMBO Journal*, 21(21), 5635 – 5644.

Hanif, R., Pittas, A., Feng, Y., Koutsos, M. I., Qiao, L., Staiano-Coico, L., Shiff, S. I., and Rigas, B., (1996), 'Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway', *Biochemical Pharmacology*, 52(2), 237 – 245.

Harris, R. C., McKanna, J. A., Akai, Y., Jacobson, H. R., Dubois, R. N., and Breyer, M. D., (1994), 'Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction', *Journal of Clinical Investigation*, 94(6), 2504 – 2510.

Harris, R. C., and Breyer, M. D., (2001), 'Physiological regulation of cyclooxygenase-2 in the kidney', *American Journal of Physiology: Renal Physiology*, 281(1), F1 – F11.

Harris, R. E., Beebe-Donk, J., and Schuller, H. M., (2002), 'Chemoprevention of Lung Cancer by Non-Steroidal Anti-Inflammatory Drugs Among Cigarette Smokers', *Oncology Reports*, 9(4), 693 – 695.

Harris, R. E., Beebe-Donk, J., and Alshafie, G. A., (2011), 'Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors: Final results of a case control study', *International Journal of Cancer Prevention*, 3(1), 113 – 124.

Hasegawa, U., Wang, T., Chen, J. J. Y., Uyama, H., and van der Vlies, A. J., (2016), 'Furoxan-Bearing Micelles for Nitric Oxide Delivery', *Macromolecular Bioscience*, 16(7), 1009 – 1018.

Heckert, E. G., Karakoti, A. S., Seal, S., and Self, W. T., (2008), 'The role of cerium redox state in the SOD mimetic activity of nanoceria', *Biomaterials*, 29(18), 2705 – 2709.

Hippisley-Cox, J., and Coupland, C., (2005), 'Risk of myocardial infarction in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis', *The BMJ*, 330(7504):1366.

Hoshino, T., Tsutsumi, S., Tomisato, W., Hwang, H. J., Tsuchiya, T., and Mizushima, T., (2003), 'Prostaglandin E2 Protects Gastric Mucosal Cells from Apoptosis via EP2 and EP4 Receptor Activation', *Journal of Biological Chemistry*, 278, 12752 – 12758.

Hossain, M. A., Kim, D. H., Jang, J. Y., Kang, Y. J., Yoon, J. H., Moon, J. O., Chung, H. Y., Kim, G. Y., Choi, Y. H., Copple, B. L., and Kim, N. D., (2012), 'Aspirin induces apoptosis in vitro and inhibits tumor growth of human hepatocellular carcinoma cells in a nude mouse xenograft model', *International Journal of Oncology*, 40(4), 1298 – 1304.

Hsu, A. L., Ching, T. T., Wang, D. S., Song, X., Ragnekar, V. M., and Chen, C. S., (2000), 'The cyclooxygenase-2 Inhibitor Celecoxib Induces Apoptosis by Blocking Akt Activation in Human Prostate Cancer Cells Independently of Bcl-2', *The Journal of Biological Chemistry*, 275(15), 11397 – 11403.

Huang, B., Zhang, J., Hou, J., and Chen, C., (2003), 'Free radical scavenging efficiency of Nano-Se in vitro', *Free Radical Biology and Medicine*, 35(7), 805 – 813.

Huang, L., Mackenzie, G. G., Ouyang, N., Sun, Y., Xie, G., Johnson, F., Komninou, D., and Rigas, B., (2011), 'The novel phospho-non-steroidal anti-inflammatory drugs, OXT-328, MDC-22 and MDC-917, inhibit adjuvant-induced arthritis in rats', *British Journal of Pharmacology*, 162(7), 1521 – 1533.

Iczkowski, K. A., and Pantazis, C. G., (2003), 'Overexpression of NSAID-activated Gene Product in Prostate Cancer', *International Journal of Surgical Pathology*, 11(3), 159 – 166.

Jiang, T., Xu, G., Chen, G., Zheng, Y., He, B., and Ghu, Z., (2020), 'Progress in transdermal drug delivery systems for cancer therapy', *Nano Research*, https://doi.org/10.1007/s12274-020-2664-5.

Jin, J., (2015), 'Nonsteroidal Anti-inflammatory Drugs', *Journal of the American Medical Association*, 314(10), 1084.

Kang, S. U., Lee, B. S., Lee, S. H., Baek, S. J., Shin, Y. S., and Kim, C. H., (2013), 'Expression of NSAID-activated gene-1 by EGCG in head and neck cancer: involvement of ATM-dependent p53 expression', *Journal of Nutritional Biochemistry*, 24(6), 986 – 989.

Kargman, S., Charleson, S., Cartwright, M., Frank, J., Riendeau, D., Mancini, J., Evans, J., and O'Neill, G., (1996), 'Characterization of Prostaglandin G/H Synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts', *Gastroenterology*, 111(2), 445 – 454.

Kawahara, K., Hohjoh, H., Inazumi, T., Tsuchiya, S., and Sugimoto, Y., (2015), 'Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors', *Biochimica et Biophysica Acta*, 1851(4), 414 – 421.

Kawahara, T., Ishiguro, H., Hoshino, K., Teranishi, J., Miyoshi, Y., Kubota, Y., and Uemura, H., (2010), 'Analysis of NSAID-Activated Gene 1 Expression in Prostate Cancer', *Urologia Internationalis*, 84(2), 198 – 202.

Ke, C. J., Chiang, W. L., Liao, Z. X., Chen, H. L., Lai, P. S., Sun, J. S., and Sung, H. W., (2013), 'Real-time visualization of pH-responsive PLGA hollow particles containing a gas-generating agent targeted for acidic organelles for overcoming multi-drug resistance', *Biomaterials*, 34(1), 1 – 10.

Khan, I., Saeed, K., and Khan, I., (2019), 'Nanoparticles: Properties, applications and toxicities', *Arabian Journal of Chemistry*, 12(7), 908 – 931.

Kim, M. S., Kim, J. E., Huang, Z., Chen, H., Langfald, A., Lubet, R. A., Grubbs, C. J., Dong, Z., and Bode, A., (2014), 'Naproxen induces cell cycle arrest and apoptosis in human urinary bladder cancer cell lines and chemically induced cancers by targeting PI3-K', *Cancer Prevention Research*, 7(2), 236 – 245.

Klabunde, T., Petrassi, H. M., Oza, V. B., Raman, P., Kelly, J. W., and Sacchettini, J., C., (2000), 'Rational design of potent human transthyretin amyloid disease inhibitors', *Nature Structural Biology*, 7(4), 312 – 321.

Kobayashi, S., and Müllen, K. (Eds.), (2004), '*Encyclopedia of Polymeric Nanomaterials*', Springer, Berlin, p. 1.

Koki, A. T., and Masferrer, J. L., (2002), 'Celecoxib: a specific COX-2 inhibitor with anticancer properties', *Cancer Control*, 9(2) (Supplement), 28 – 35.

Koklic, T., (2014), 'Perifosine induced release of contents of trans cell-barrier transport efficient liposomes', *Chemistry and Physics of Lipids*, 183, 50 – 59.

Komhoff, M., Grone, H. J., Klein, T., Seyberth, H. W., and Nursing, R. M., (1997), 'Localization of cyclooxygenase-1 and -2 in adult and fetal human kidney: implication for renal function', *American Journal of Physiology: Renal Physiology*, 272(4), F460 – F468.

Kulp, H. S., Yang, Y. T., Hung, C. C., Chen, K. F., Lai, J. P., Tseng, P. H., Fowble, J. W., Ward, P. J., and Chen, C. S., (2004), '3-phosphoinositide-dependent Protein kinase-1/Akt Signaling Represents a Major cyclooxygenase-2-independent Target for Celecoxib in Prostate Cancer Cells', *Cancer Research*, 64(4), 1444 – 1451.

Kumar, R., Siril, P. F., and Javid, F., (2016), 'Unusual anti-leukemia activity of nanoformulated naproxen and other non-steroidal anti-inflammatory drugs', *Materials Science and Engineering: C, Materials for Biological Applications*, 69, 1335 – 1344.

Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., and Muller, R. N., (2008), 'Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications', *Chemical Reviews*, 108(6), 2064 – 2110.

Lawson, R. N., and Chughtai, M. S., (1963), 'Breast Cancer and Body Temperature', *Canadian Medical Association Journal*, 88(2), 68 – 70.

Layek, B., and Singh, J., (2013), 'Amino acid grafted chitosan for high performance gene delivery: comparison of amino acid hydrophobicity on vector and polyplex characteristics', *Biomacromolecules*, 14(2), 485 – 494.

Lee, B. S., Yoon, C. W., Osipov, A., Moghavem, N., Nwachokor, D., Amatya, R., Na, R., Pantoja, J. L., Pham, M. D., Black, K. L., and Yu, J., S., (2011), 'Nanoprodrugs of NSAIDs: Preparation and Characterization of Flufenamic Acid Nanoprodrugs', *Journal of Drug Delivery*, 2011, 980720.

Li, H., Huo, M., Zhou, J., Dai, Y., Deng, Y., Shi, X., and Masoud, J., (2010), 'Enhanced Oral Absorption of Paclitaxel in N-Deoxycholic Acid-N, O-Hydroxyethyl Chitosan Micellar System', *Journal of Pharmaceutical Sciences*, 99(11), 4543 – 4553.

Li, X., Chen, Y., Wang, M., Ma, Y., Xia, W., and Gu, H., (2013), 'A mesoporous silica nanoparticle – PEI – fusogenic peptide system for siRNA delivery in cancer therapy', *Biomaterials*, 34(4), 1391 – 1401.

Liu, W., Park, Y., Purdue, M. P., Giovannucci, E., and Cho, E., (2013), 'A large cohort study of nonsteroidal anti-inflammatory drugs and renal cell carcinoma incidence in the National Institutes of Health–AARP Diet and Health Study', *Cancer Causes & Control*, 24(10), 1865 – 1873.

Lubet, R. A., Scheiman, J. M., Bode, A., White, J., Minasian, L., Juliana, M. M., Boring, D. L., Steele, V. E., and Grubbs, C. J., (2015), 'Prevention of chemically induced urinary bladder cancers by naproxen: protocols to reduce gastric toxicity in humans do not alter preventive efficacy', *Cancer Prevention Research*, 8(4), 296 – 302.

Malhotra, B. D., and Ali, A., (2017), '*Nanomaterials for Biosensors: Fundamentals and Applications*', 1st Edition, Elsevier, p. 1 – 77.

Mansouri, D., McMillan, D. C., Roxburgh, C. S. D., Crighton, E. M., and Horgan, P. G., (2013), 'The Impact of Aspirin, Statins and ACE-inhibitors on the Presentation of Colorectal Neoplasia in a Colorectal Cancer Screening Programme', *British Journal of Cancer*, 109(1), 249 – 256.

Marcheselli, V. L., and Bazan, N. G., (1996), ‘Sustained induction of prostaglandin endoperoxide synthase-2 by seizures in hippocampus. Inhibition by a platelet-activating factor antagonist’, *The Journal of Biological Chemistry*, 271(40), 24794 – 24799.

Marques, J. G., Gaspar, V. M., Costa, E., Paquete, C. M., and Correia, I. J., (2014), ‘Synthesis and characterization of micelles as carriers of non-steroidal anti-inflammatory drugs (NSAID) for application in breast cancer therapy’, *Colloids and Surfaces B: Biointerfaces*, 113, 375 – 383.

Mbonye, U. R., Yuan, C., Harris, C. E., Sidhu, R. S., Song, I., Arakawa, T., and Smith, W. L., (2008), ‘Two distinct pathways for cyclooxygenase-2 protein degradation’, *The Journal of Biological Chemistry*, 283(13), 8611 – 8623.

Mizushima, T., (2010), ‘Molecular Mechanism for Various Pharmacological Activities of NSAIDs’, *Pharmaceuticals*, 3, 1614 – 1636.

Najafzadeh, M., Baumgartner, A., Gopalan, R., Davies, J. B., Wright, A., Reynolds, P. D., and Anderson, D., (2012), ‘In vitro sensitivities to UVA of lymphocytes from patients with colon and melanoma cancers and precancerous states in the micronucleus and the Comet assays’, *Mutagenesis*, 27(3), 351 – 357.

Najafzadeh, M., Normington, C., Jacob, B. K., Isreb, M., Gopalan, R. C., and Anderson, D., (2016), ‘DNA Damage in Healthy Individuals and Respiratory Patients after Treating Whole Blood In vitro with the Bulk and Nano Forms of NSAIDs’, *Frontiers in Molecular Biosciences*, 3:50, eCollection 2016.

Nakase, I., Konishi, Y., Ueda, M., Saji, H., and Futaki, S., (2012), ‘Accumulation of arginine-rich cell-penetrating peptides in tumors and the potential of anticancer drug delivery *in vivo*’, *Journal of Controlled Release*, 159(2), 181 – 188.

Nie, T., Wong, C. C., Alston, N., Aro, P., Constantinides, P. P., and Rigas, B., (2011), ‘Phospho-ibuprofen (MDC-917) incorporated in nanocarriers: anti-cancer activity in vitro and in vivo’, *British Journal of Pharmacology*, 166(3), 991 – 1001.

Otsuka, H., Nagasaki, Y., and Kataoka, K., (2003), ‘PEGylated nanoparticles for biological and pharmaceutical applications’, *Advanced Drug Delivery Reviews*, 55(3), 403 – 419.

Patel, M. I., Subbaramaiah, K., Du, B., Chang, M., Yang, P., Newman, R. A., Cordon-Cardo, C., Thaler, H. T., and Dannenberg, A. J., (2005), 'Celecoxib Inhibits Prostate Cancer Growth: Evidence of a cyclooxygenase-2-independent Mechanism', *Clinical Cancer Research*, 11(5), 1999 – 2007.

Piazza, G. A., Alberts, D. S., Hixson, L. J., Paranka, N. S., Li, H., Finn, T., Bogert, C., Guillen, J. M., Brendel, K., Gross, P. H., Sperl, G., Ritchie, J., Burt, R. W., Ellsworth, L., Ahnen, D. J., and Pamukcu, R., (1997), 'Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostaglandin levels', *Cancer Research*, 57(14), 2909 – 2915.

Prakash, P., Sayyed-Ahmad, A., Zhou, Y., Volk, D. E., Gorenstein, D. G., Dial, E., Lichtenberger, L. M., Gorfe, A. A., (2012), 'Aggregation behavior of ibuprofen, cholic acid and dodecylphosphocholine micelles', *Biochimica et Biophysica Acta*, 1818(12), 3040 – 3047.

Reddy, B. S., Kawamori, T., Lubet, R. A., Steele, V. E., Kelloff, G. J., and Rao, C. V., (1999), 'Chemopreventive efficacy of sulindac sulfone against colon cancer depends on time of administration during carcinogenic process', *Cancer Research*, 59(14), 3387 – 3391.

Rekharsky, M. V., and Inoue, Y., (1998), 'NMR Studies of Cyclodextrins and Cyclodextrin Complexes', *Chemical Reviews*, 98(5), 1755 – 1786.

Retsky, M., Rogers, R., Demicheli, R., Hrushesky, W. J., Gukas, I., Vaidya, J. S., Baum, M., Forget, P., Dekock, M., and Pachmann, K., (2012), 'NSAID Analgesic Ketorolac Used Perioperatively May Suppress Early Breast Cancer Relapse: Particular Relevance to Triple Negative Subgroup', *Breast Cancer Research and Treatment*, 134(2), 881 – 888.

Rigas, B., and Kashfi, K., (2004), 'Nitric-oxide-donating NSAIDs as agents for cancer prevention', *Trends in Molecular Medicine*, 10(7), 324 – 330.

Ristimäki, A., Honkanen, N., Jänkälä, H., Sipponen, P., and Härkönen, M., (1997), 'Expression of cyclooxygenase-2 in human gastric carcinoma', *Cancer Research*, 57(7), 1276 – 1280.

Rolland, P. H., Martin, P. M., Jacquemier, J., Rolland, A. M., and Toga, M., (1980), 'Prostaglandin in Human Breast Cancer: Evidence Suggesting That an Elevated Prostaglandin Production Is a Marker of High Metastatic Potential for Neoplastic Cells', *Journal of the National Cancer Institute*, 64(5), 1061 – 1070.

Rouzer, C. A., and Marnett, L. J., (2003), 'Mechanism of free radical oxygenation of polyunsaturated fatty acids by cyclooxygenases', *Chemical Reviews*, 103(6), 2239 – 2304.

Rouzer, C. A., and Marnett, L. J., (2009), 'Cyclooxygenases: structural and functional insights', *Journal of Lipid Research*, 50(Supplement), S29 – S34.

Rössler, A., Vandermeulen, G. W. M., and Klok, H. A., (2012), 'Advanced drug delivery devices via self-assembly of amphiphilic block copolymers', *Advanced Drug Delivery Reviews*, 64(Supplement), 270 – 279.

Sánchez, P., Peñarroja, R., Gallegos, F., Bravo, J. L., Rojas, E., and Benítez-Bribiesca, (2004), 'DNA damage in peripheral lymphocytes of untreated breast cancer patients', *Archives of Medical Research*, 35(6), 480 – 483.

Sestakova, Z., Kalavska, K., Hurbanova, L., Jurkovicova, D., Gursky, J., Chovanec, M., Svetlovska, D., Miskovska, V., Obertova, J., Palacka, P., Rejlekova, K., Sycova-Mila, Z., Cingelova, S., Spanik, S., Mardiak, J., Chovanec, M., and Mego, M., (2016), 'The prognostic value of DNA damage level in peripheral blood lymphocytes of chemotherapy-naïve patients with germ cell cancer', *Oncotarget*, 7(46), 75996 – 76005.

Shang, L., Najafzadeh, M., and Anderson, D., (2014), 'Whole-cell Currents Recording from Ion Channels in Human Lymphocytes Treated with Anti-inflammatory Drugs in Nanoparticles Forms', *International Journal of Computer, Consumer and Control (IJ3C)*, 3(3), 11 – 20.

Sharpe, C. R., Collet, J. P., McNutt, M., Belzile, E., Boivin, J. F., and Hanley, J. A., (2000), 'Nested case–control study of the effects of nonsteroidal anti-inflammatory drugs on breast cancer risk and stage', *British Journal of Cancer*, 83(1), 112 – 120.

Sirois, J., Sayasith, K., Brown, K. A., Stock, A. E., Bouchard, N., and Doré, M., (2004), 'Cyclooxygenase-2 and its role in ovulation: a 2004 account', *Human Reproduction Update*, 10(5), 373 – 385.

Siskou, I. C., Rekka, E. A., Kourounakis, A. P., Chrysselis, M. C., Tsiakitzis, K., and Kourounakis, P. N., (2007), 'Design and study of some novel ibuprofen derivatives with potential nootropic and neuroprotective properties', *Bioorganic and Medicinal Chemistry*, 15(2), 951 – 961.

Smalley, W. E., Ray, W. A., Daugherty, J. R., and Griffin, M. R., (1995), 'Nonsteroidal anti-inflammatory drugs and the incidence of hospitalizations for peptic ulcer disease in elderly persons', *American Journal of Epidemiology*, 141(6), 539 – 545.

Smith, W. L., (1986), 'Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells', *Annual Review of Physiology*, 48, 251 – 262.

Smith, W. L., DeWitt, D. L., and Garavito, R. M., (2000), 'Cyclooxygenases: structural, cellular, and molecular biology', *Annual Review of Biochemistry*, 69, 145 – 182.

Srinivas, S., and Feldman, D., (2009), 'A phase II trial of calcitriol and naproxen in recurrent prostate cancer', *Anticancer Research*, 29(9), 3605 – 3610.

Stavreus-Evers, A., Koraen, L., Scott, J. E., Zhang, P., and Westlund, P., (2005), 'Distribution of cyclooxygenase-1, cyclooxygenase-2, and cytosolic phospholipase A2 in the luteal phase human endometrium and ovary', *Fertility & Sterility*, 83(1), 156 – 162.

Stephan, C., Rittenhouse, H., Hu, X., Cammann, H., and Jung, K., (2014), 'Prostate-Specific Antigen (PSA) Screening and New Biomarkers for Prostate Cancer (PCa)', *eJIFCC: Electronic Journal of the International Federation of Clinical Chemistry and Laboratory Medicine*, 25(1), 55 – 78.

Tammara, V. K., Narurkar, M. M., Crider, A. M., and Khan, M. A., (1993), 'Synthesis and evaluation of morpholinoalkyl ester prodrugs of indomethacin and naproxen', *Pharmaceutical Research*, 10(8), 1191 – 1199.

Tan, M. L., Choong, P. F. M., and Dass, C. R., (2010), 'Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery', *Peptides*, 31(1), 184 – 193.

Tian, W., Zhao, Y., Liu, S., and Li, X., (2010), 'Meta-analysis on the Relationship between Nonsteroidal Anti-Inflammatory Drug Use and Gastric Cancer', *European Journal of Cancer Prevention*, 19(4), 288 – 298.

Trautman, M. S., Edwin, S. S., Collmer, D., Dudley, D. J., Simmons, D., and Mitchell, D., (1996), 'Prostaglandin H synthase-2 in human gestational tissues: Regulation in amnion', *Placenta*, 17(4), 239 – 245.

Tsujii, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M., and DuBois, R. N., (1998), 'Cyclooxygenase Regulates Angiogenesis Induced by Colon Cancer Cells', *Cell*, 93(5), 705 – 716.

Uekama, K., Hirayama, F., and Irie T., (1998), 'Cyclodextrin Drug Carrier Systems', *Chemical Reviews*, 98(5), 2045 – 2076.

Van Antwerpen, P., Dufrasne, F., Lequeux, M., Boudjeltia, K. Z., Lessgyer, I., Babar, S., Moreau, P., Moguilevski, N., Vanhaeverbeek, M., Ducobu, Jean, and Nève, J., (2007), 'Inhibition of the myeloperoxidase chlorinating activity by non-steroidal anti-inflammatory drugs: Flufenamic acid and its 5-chloro-derivative directly interact with a recombinant human myeloperoxidase to inhibit the synthesis of hypochlorous acid', *European Journal of Pharmacology*, 570(1-3), 235 – 243.

Vane, J. R., and Botting, R. M., (1998), 'Anti-inflammatory drugs and their mechanism of action', *Inflammation Research*, 47(Supplement), S78 – S87.

Vane, J., Botting, J., and Botting, R. (Eds.), (2015), '*Improved Non-Steroid Anti-inflammatory Drugs: COX-2 Enzyme Inhibitors*', Lancaster, London: Kluwer Academic Publishers and William Harvey Press, p. 167 – 188.

Vázquez-González, M. L., Bernad, R., Calpena, A. C., Domènech, O., Montero, M. T., and Hernández-Borrell, J., (2014), 'Improving ex vivo skin permeation of non-steroidal anti-inflammatory drugs: enhancing extemporaneous transformation of liposomes into planar lipid bilayers', *International Journal of Pharmaceutics*, 461(1-2), 427 – 436.

Vázquez-González, M. L., Botet-Carreras, A., Domènech, O., Montero, M. T., and Borrell, J. H., (2019), 'Planar lipid bilayers formed from thermodynamically-optimized liposomes as new featured carriers for drug delivery systems through human skin', *International Journal of Pharmaceutics*, 563, 1 – 8.

Velázquez, C. A., Praveen Rao, P. N., Citro, M. L., Keefer, L. K., and Knaus, E. E., (2007), 'O2-acetoxymethyl-protected diazeniumdiolate-based NSAIDs (NONO-NSAIDs): synthesis, nitric oxide release, and biological evaluation studies', *Bioorganic and Medicinal Chemistry*, 15(14), 4767 – 4774.

Verma, A., and Stellacci, F., (2010), 'Effect of surface properties on nanoparticle-cell interactions', *Small*, 6(1), 12 – 21.

Wang, P. G., Cai, T. B., and Taniguchi, N. (Eds.), (2005), '*Nitric Oxide Donors: For Pharmaceutical and Biological Applications*', Wiley-VCH Weinheim, Verlag GmbH & Co. KGaA, p. 131.

Whittle, B. J. R., and Vane, J. R., (1987), 'Prostanoids as regulators of gastrointestinal function', in Johnston, L. R. (Ed.), '*Physiology of the Gastrointestinal Tract*', Volume 1, 2nd Edition, New York: Raven Press, 143 – 180.

Whittle, B. J. R., (2003), 'Gastrointestinal effects of nonsteroidal anti-inflammatory drugs', *Fundamental and Clinical Pharmacology*, 17(3), 301 – 313.

Williams, J. L., Borgo, S., Hasan, I., Castillo, E., Traganos, F., and Rigas, B., (2001), 'Nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) alter the kinetics of human colon cancer cell lines more effectively than traditional NSAIDs: implications for colon cancer chemoprevention', *Cancer Research*, 61(8), 3285 – 3289.

Wojtkowiak, J. W., Verduzco, D., Schramm, K. J., and Gillies, R. J., (2011), 'Drug resistance and cellular adaptation to tumor acidic pH microenvironment', *Molecular Pharmacology*, 8(6), 2032 – 2038.

Wolfe, M. M., Lichtenstein, D. R., and Singh, G., (1999), 'Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs', *The New England Journal of Medicine*, 340(24), 1888 – 1899.

Wongrakpanich, S., Wongrakpanich, A., Melhado, K., and Rangaswami, J., (2018), ‘A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly’, Aging and Disease, 9(1), 143 – 150.

Xie, G., Sun, Y., Nie, T., Mackenzie, G. G., Huang, L., Kopelovich, L., Komninou, D., and Rigas, B., (2011), ‘Phospho-ibuprofen (MDC-917) is a novel agent against colon cancer: efficacy, metabolism, and pharmacokinetics in mouse models’, *The Journal of Pharmacology and Experimental Therapeutics*, 337(3), 876 – 886.

Yamagata, K., Andreasson, K. I., Kaufmann, W. E., Barnes, C. A., and Worley, P. F., (1993), ‘Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids’, *Neuron*, 11(2), 371 – 386.

Yermakova, A. V., and O’Banion, M. K., (2001), ‘Downregulation of neuronal cyclooxygenase-2 expression in end stage Alzheimer's disease’, *Neurobiology of Aging*, 22(6), 826 – 836.

Zhang, X., Morham, S. G., Langenbach, R. and Young, D. A., (1999), ‘Malignant transformation and antineoplastic actions of nonsteroidal antiinflammatory drugs (NSAIDs) on cyclooxygenase-null embryo fibroblasts’, *Journal of Experimental Medicine*, 190(4), 451 – 460.

Zhang, Y., Coogan, P. F., Palmer, J. R., Strom, B. L., and Rosenberg, L., (2005), ‘Use of Nonsteroidal Antiinflammatory Drugs and Risk of Breast Cancer: The Case-Control Surveillance Study Revisited’, *American Journal of Epidemiology*, 162(2), 165 – 170.

Zhao, J., Guo, S., Sun, Y., Yin, R., and Wang, Z., (2012), ‘Nitric Oxide Donor-Based Platinum Complexes as Potential Anticancer Agents’, *Chemistry – A European Journal*, 18(45), 14276 – 14281.

Zhao, M., Huang, Y., Chen, Y., Xu, J., Li, S., and Guo, X., (2016), ‘PEG-Fmoc-Ibuprofen Conjugate as a Dual Functional Nanomicellar Carrier for Paclitaxel’, *Bioconjugate Chemistry*, 27(9), 2198 – 2205.

Zidar, N., Odar, K., Glavac, D., Jerse, M., Zupanc, T., and Stajer, D., (2009), ‘Cyclooxygenase in normal human tissues--is COX-1 really a constitutive isoform, and COX-2 an inducible isoform?’, *Journal of Cellular and Molecular Medicine*, 13(9B), 3753 – 3763.