Chapter 20

Low Molecular Weight Hyaluronic Acid Fe_3O_4 Nanoparticles: Biological Assessment of Their Ability to Target Breast Cancer Cells

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ABSTRACT

This study involved superparamagnetic Fe_3O_4 nanoparticles (Fe_3O_4 NPs) with low-molecular-weight hyaluronic acid (LMWHA). A comprehensive analysis was conducted on the size distribution, zeta potential, viscosity, thermogravimetric, and paramagnetic characteristics of LMWHA-Fe_3O_4 nanoparticles. The MCF7 breast cancer cell line was used for cellular experiments. In addition, the researchers employed the thiocyanate method and time-of-flight secondary ion mass spectrometry (TOF-SIMS) to analyze the properties and ability of LMWHA-Fe_3O_4 NPs to target MCF7 breast cancer cells. The experiment revealed that the LMWHA-Fe_3O_4 NPs had a prominent superparamagnetic property and may be easily injected due to their low viscosity. Furthermore, the outcomes of the in vitro experiment revealed that the nanoparticles exhibited a strong capacity to specifically target cancer cells while exhibiting minimal damage. The results suggest that LMWHA-Fe_3O_4 nanoparticles show potential as an injectable drug for enhancing magnetic resonance imaging (MRI) and treatment of hyperthermia in breast cancer.

KEYWORDS
Iron Oxide, Nanoparticles, Hyaluronic Acid, Alternatives, Breast Cancer

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INTRODUCTION

Cancer comprises a group of diseases described by uncontrolled cell proliferation, with the capacity for these aberrant cells to infiltrate and spread to other body regions. Classification of cancer is based on tumor cells that is classified that include, carcinoma, sarcoma, lymphoma, and germ cell tumors. Carcinoma specifically refers to malignancies that originate from epithelial cells and include a wide range of tumors such as breast, prostate, lung, pancreatic, and colon cancers (Varricho, 2004). Cancer is a hereditary illness linked to unchecked cell division and inhibition of cell death (Yahya and Alqadhi, 2021).

Around 9.6 million deaths worldwide are attributed to cancer, which is still the leading cause of mortality due to cancer, due to a lack of efficient early detection techniques (Miranda et al., 2020). In 2018, 2 million new cases of breast cancer (BC) were identified, making it the most common invasive malignancy diagnosed in women worldwide (Mirza et al., 2021). However, it is thought to be curable if detected early, particularly before metastasis (Liyanage et al., 2019).

In consideration of the adverse effects that different types of cancer cause, skin, and lung cancer rank as the most prevalent cancers globally. Furthermore, BC is the most frequent type of cancer in women, making up over 30% of all cancer occurrences (Liyanage et al., 2019). On the contrary, BC in men constitutes merely 1% of all malignant breast neoplasms (Fentiman et al., 2006). Additionally, in comparison to women, men typically receive a diagnosis of BC at a more advanced age, around 67 years (Medeira et al., 2011). Despite being the most prevalent type of cancer in women, if detected early enough, it is thought to be curable (Keivit et al., 2015; Seigel et al., 2017).

Like other cancers, breast cancer is traditionally treated with surgery, chemotherapy, and radiation. These treatments' main objective is to remove tumors while extending the patients' lives. However, advanced and metastatic cancers pose a challenge to these standard approaches in terms of medication resistance and tumor recurrence. For example, when a tumor recurs and spreads to other organs such as the liver, lung, and bone, surgery is ineffective. In contrast,

chemotherapy aims to utilize cytotoxic chemotherapeutic drugs, administered either after or independently of surgery, to impede the division and growth of tumor cells. Radiotherapy, on the other hand, entails the delivery of potent energy waves to disrupt tumor cell division, leading to the reduction or elimination of tumors. While both chemotherapy and radiotherapy are potent cancer treatment methods designed to enhance survival rates, they may also result in acute and long-term adverse effects on the healthy organs of patients (Thati et al., 2010; Dao et al., 2012). For example, a drug known as a monoclonal antibody used in cancer treatment has demonstrated toxicity assistance with cardiac dysfunction when administered over a long period (Zeglinski et al., 2011).

Breast cancer is usually a result of malignant breast cells spreading out of control (Barzaman et al., 2020). The most common cancer in women, breast cancer requires special care because it does not respond well to standard treatment (Miller et al., 2019). Breast cancer can be treated in a variety of ways, with several side effects, such as surgery, chemotherapy, radiation, and hormone treatment (Valencia et al., 2017; Waks and Winer, 2019). Limiting the toxicity of medications to normal cells by specifically targeting them is the optimum approach to treating cancer (Klochkov et al., 2021).

Furthermore, it is difficult to deal with the overexpression of certain proteins in tumor cells that leads to multidrug resistance. In this situation, the impact of chemotherapy is frequently significantly diminished. Radiation therapy is a type of local therapy that solely targets the tumor's exact site. However, because of the harm done to the nearby healthy tissues, adverse consequences might happen. Given the negative effects of traditional cancer treatment methods, it is imperative to look for new and effective alternatives.

Nanoparticles

According to the International Union of Pure and Applied Chemistry, nanoparticles (NPs) are tiny particles with dimensions between 1 to 100 nm (Batista et al., 2015; Kantoff et al., 2017). These particles are suitable for materials science and biology because of their unique physical characteristics, which include conductivity, stability, and optical qualities. Nanoparticles exhibit effectiveness across various fields, including medicine, pharmacy, and many others like environmental science, energy, electronics, and biomolecules (Kausar et al., 2023). This is attributed to their versatile applications in optical, biological, and electronic domains, showcasing their wide-ranging impact. They are categorized into many groups according to their characteristics, dimensions, and forms (Khan et al., 2019).

Targeted therapies stand out as the most efficient treatments for common cancers, surpassing other modalities like conventional chemotherapy. They offer advantages such as reduced side effects, enhanced viability, lower dosage needs, fewer adverse reactions, improved therapeutic indicators, and more precise, specific treatment goals (Senapati et al., 2018). Recent studies indicate that nanoparticles provide numerous advantages in tumor diagnosis and therapy. These benefits extend beyond medications and include applications in imaging agents and genes (Farokhzad et al., 2009).

Over the past 20 years, the properties of NPs have led to the development of numerous NP-based therapy approaches in clinical trials. In this ongoing study, we assessed the efficacy of nanoparticles in treating cancer, comparing their effectiveness with alternative drug delivery strategies (Khan et al., 2023).

Utilizing NPs (Nanoparticles) for Drug Delivery in the Field of Anticancer Treatment

NPs have recently been acknowledge as potential carriers for medications. Drugs' pharmacokinetic features are changed by using nanocarriers to increase their effectiveness and decrease their negative effects (Karra et al., 2012). These systems release the medications at precise locations and timings, thereby influencing the body's pharmacokinetics and the processes of drug distribution (Tiwari et al., 2012).

The special features of their structure can make therapeutic drugs work better. Drugs are released in the body, defending drug molecules, being smaller than cells, moving through biological barriers to reach specific targets, keeping drug active in bloodstream for longer, helping deliver drug to specific places, and being safe for the body (De Jong and Borm, 2008).

Over the past fifty years, there has been a substantial evolution in the diversity of nanocarriers due to various developments in the disciplines of chemistry, biology, mechanics, polymers, and physics. As a result, a variety of carriers with distinct qualities have been brought into the medical sciences (Khan et al., 2017).

Drug distribution is a method of delivering medication to the human body in the most effective manner (Shreyash et al., 2021). To produce a desired impact on the body, it entails a range of formulations, methods, and techniques, including but not limited to nanoparticles. The major goal is to get right amount of medication to the right location in the body without reducing quality in order to have a greatest possible benefit and avoid any negative side effects. The goal of methods including site-targeting and systematic toxicology is same (Blugarten, 1915). Although the available nanohybrids, particularly the ones used to treat malignancies, have prolonged bloodstream residence periods, extravasation (leakage) of nanoparticles is favored (Wang et al., 2018). The ability of nanoparticles to split into different fine sized inorganic particles is another advantage of using them as shown in Fig. 1.1.

When a nanomedicine come in contact with white blood cells (WBCs), namely leucocytes, they determine whether the medicine will be absorbed or whether an immune reaction will be triggered against it (Diaz et al., 2008). Before they may be examined with TEM (transmission electron microscope) imaging, the observation necessitates several treatments which may change the organelles (Gupta et al., 2005).

Different outcomes are elicited by nanoparticles of varying sizes. Mesoporous silica nanoparticles (MSNs) with round

shape and size of 100nm could not considerably break the membrane of red blood cells (RBCs), while bigger particles, measuring 600nm, induced a considerable disruption that might potentially result in hemolysis (Zhao et al., 2011).

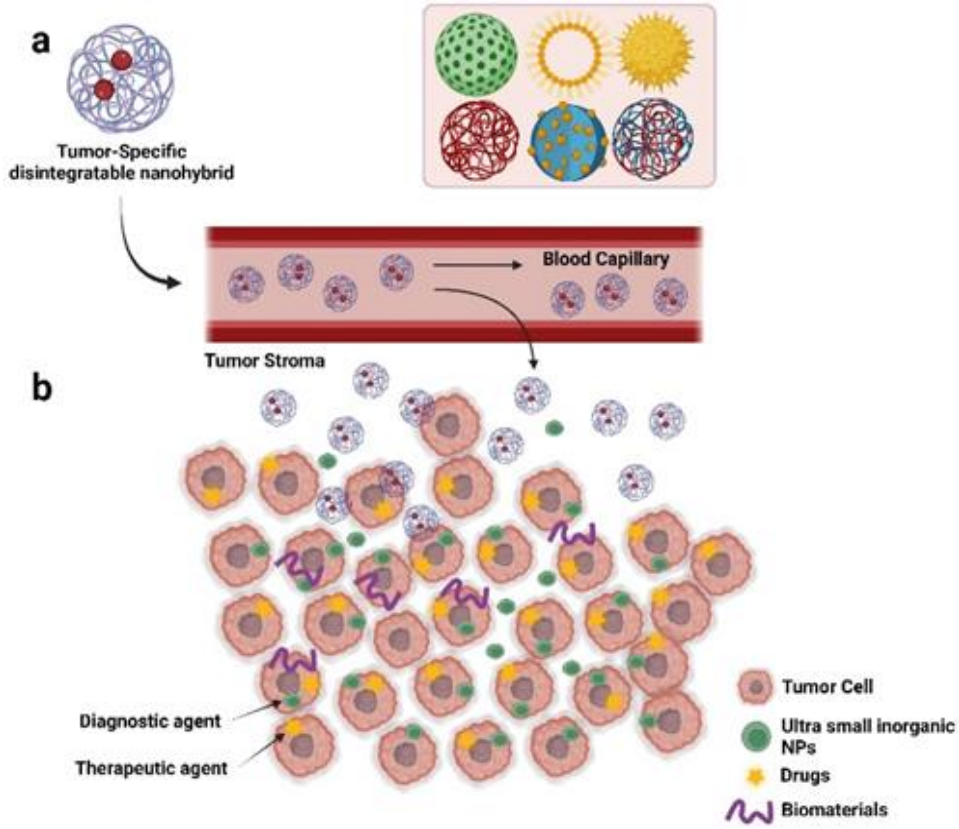

Fig. 1.1: (a) Nano hybrids, which are made of tiny inorganic nanoparticles, are used for effective cancer treatment. (b) Nano hybrids are utilized in cancer therapy

The goal of using nanoparticles for medication distribution is to make sure that the pharmaceuticals are absorbed more by cancer cells that are targeted and less by healthy cells. To achieve this, targeting agents are used. Functionalization of nanoparticles has an impact on how they interact with cell membranes. Individual nanoparticles cross the membrane more easily than when they are clumped together. Over time, methods for assessing the efficiency of nanoparticles in cancer treatment have greatly improved. One study introduced the enhanced permeability and retention (EPR) effect. This concept came from research evaluating how well a protein polymer conjugate worked in preventing cancer (Matsumura et al., 1986). This investigation revealed that the conjugate accumulates more in tumor tissues compared to free proteins. The EPR effect (Fang et al., 2011) leads to a major increase in number of tumor cells. Figure 1.2 shows how the EPR effect helps nanoparticles enter the tumor environment and enhances their therapeutic impact.

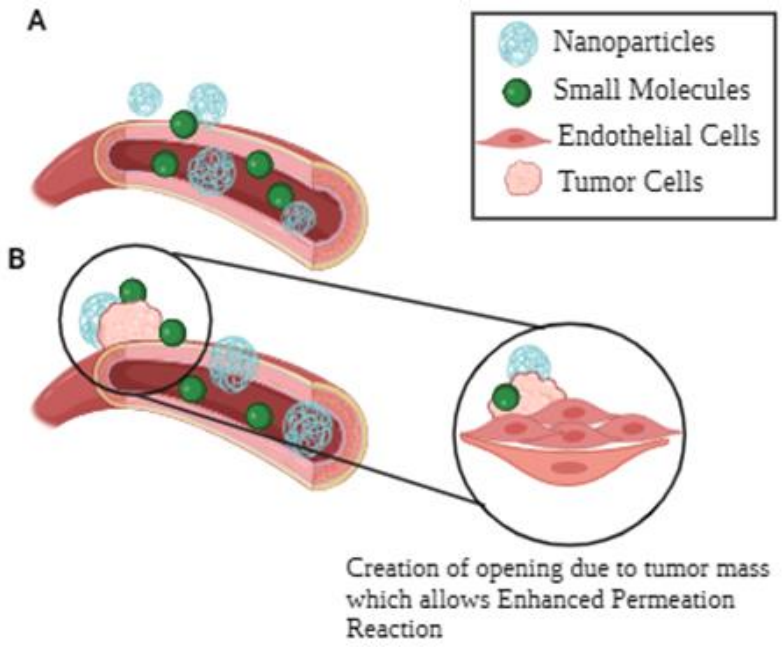

Fig. 1.2: Drug loading mechanism in a tumor cell (A) Cells in normal state. (B) Cells that have tumors develop openings that let nanoparticles move out of the blood vessels. Hyaluronic Acid

Hyaluronic acid, often known as hyaluronan or HA, is a glycosaminoglycan that is not sulfated and constitutes a significant component of the extracellular matrix. It is made up of β-N-acetylglucosamine (GIcNAc)-(1-3)-β-glucoronic acid (GlcA)-(1-4) repeating units. An increasing number of studies suggest that HA is involved in a number of biological processes, including cell adhesion, migration, and proliferation (Viola et al., 2015; Liang et al., 2016).

HA- mediated motility receptors (RHAMM), toll-like receptors (TLRs), lymphatic vessel endothelial hyaluronan receptors (LYVE1), and cluster of differentiation 44 (CD44) are cell surface receptors that have been found to intact with HA (Hardwick et al., 1992; Banerji et al., 1999; Calikoglu et al., 2003; Tesar et al., 2006). Recent studies has shown that HA plays a vital role in the formation, dissemination, metastasis, and progression of cancer (Bharadwaj et al., 2007; Kothapalli et al., 2008; Weigel et al., 2017).

Breast cancer is the most frequent type of cancer among women worldwide, accounting for 25% of all cases (Nave et al., 2006; Seigel et al., 2018). Because the cancer cells in triple-negative breast cancer (TNBC) do not express HER2, progesterone receptors, or estrogen receptors, it is a high-grade, aggressive form of breast cancer that frequently has a high patient death rate (Heldin et al., 2013). Numerous investigations have shown that HA controls the development and invasions of tumor cells both in vivo and in vitro (Bourguignon et al., 2010; Tolg et al., 2014; Yang et al., 2015).

The naturally occurring polysaccharide HA binds attractively to CD44 receptors that are overexpressed on a variety of malignant cells. An increasing body of studies suggests that HA oligosaccharides (HAOs) demonstrate unique biological effects not found in native hyaluronic acid. Additionally, research indicates that the bioactivity of HAOs differs from that of HA, particularly in the context of cancer advancements. It has been observed that HA oligomers prevent tumor growth in vivo (Zeng et al., 1998).

Hyaluronic acid (HA) is a glycosaminoglycan biopolymer that is mostly found in biological creatures' connective, epidermal, neural, and joint tissues (Fraser et al., 1997). Additionally, it acts as the primary constituent of the extracellular matrix (Toole and B.P., 2009). In medical contexts, HA finds frequent application in addressing osteoarthritis and healing skin wounds. Moreover, due to its ability to readily attach to the CD44 receptors found on tumor cells (Holmes et al., 1988; Mattheolabakis et al., 2015), HA is employed as a material for targeting tumors (Kahmann et al., 2000; Toole and B.P., 2009).

HA maintains exceptional viscoelasticity even after absorbing water, making it valuable for retaining skin moisture and managing osteoarthritis (Kablik et al., 2009). Nevertheless, natural HA has a high viscosity and a molecular weight of about 2000 kDa, which makes it difficult to inject into blood arteries in humans. As a result, numerous research teams have examined the physiological characteristics of low-molecular-weight-hyaluronic acid (ranging from 80 to 800kDa) (Cowman et al., 1996; Sun et al., 2011). Research indicates that low-molecular-weight hyaluronic acid (LMWHA) may have beneficial impacts on the wound healing process, immune system function, and formation of new blood vessels (Huang et al., 2019).

Hyaluronic acid (HA) and magnetic nanoparticles combined have demonstrated potential as a contrast agent for tumor magnetic resonance imaging (MRI), with a focus on CD44 receptors on tumor cell surfaces (Zhang et al., 2014). Although progress in understanding the targeting efficiency and functionality of low-molecular-weight hyaluronic acid nanoparticles (LMWHA-NPs) through in vitro and in vivo experiments, their physical properties, particularly dynamic viscoelasticity, remain unexplored. To fully harness LMWHA-NPs as an injectable MRI contrast medium, it is essential to synthesize and characterize LMWHA with precise molecular weights and reduced viscosity. Current methods for producing LMWHA involve either physical techniques (e.g., ultrasonic, ozone) or chemical approaches (e.g., enzymatic and acid degradation) to break down high-molecular-weight hyaluronic acid (HMWHA) (Zhong et al., 2019).

Fe_3O_4 Nanoparticles

Recently, significant advancements have been made in the use of Fe_3O_4 nanoparticle (Fe_3O_4 NPs) in medical sectors, including magnetic resonance imaging (MRI) (Guo et al., 2018), medication or gene administration (Cazares et al., 2017), treatment for magnetic hyperthermia (MHT) (Albarqi et al., 2019), and bimolecular separation and cleansing (Das et al., 2010). Studies have investigated how well Fe_3O_4 NPs work in magnetic hyperthermia treatment for various cancer types, including neck, head, brain and liver cancers (Jordan et al., 2006; Wang et al., 2012; Attaluri et al 2015). The underlying principle of this technique is that, when there is elevated to between 42 and 45C, malignant cells exhibit greater sensitivity to temperature rise than normal cells.

Magnetic hyperthermia therapy (MHT) involves delivering magnetic nanoparticle (MNPs) as heat bases to tumor tissue through systemic injection. Exposing the tumor tissue to high-frequency magnetic fields subsequently induces heat generation by MNPs through hysteresis losses. This thermal effect has the potential to cause damage to or eliminate cancer cells.

Iron Oxide nanoparticles ($Fe3O_4$ NPs) are highly promising due to their exceptional superparamagnetic and biocompatible properties. Importantly, NPs exhibit the phenomena of superparamagnetic, in which they become fully magnetized and do not retain any residual magnetic interaction once the external magnetic field is removed (Mahmoudi et al., 2011). Numerous biomedical applications, including drug administration, magnetofection, and hyperthermia, hold promise for these magnetic iron oxide nanoparticles. These NPs have also been demonstrated to have cytotoxicity against cancer cells in addition to these characteristics (Calero et al., 2015; Vinardell et al., 2015). On normal cells, however, Fe_3O_4 NPs exhibited little to no effect, consistent with earlier research (Sato et al., 2013).

While the mentioned research utilized experiments with cells in a lab (in vitro) and live animals (in vivo) to evaluate

how well LMWHA-NPs targeted and performed, they didn't focus on physical properties of the particles, especially their dynamic viscoelastic features. As a result, it is extremely important to synthesize and characterize LMWHA with a specific molecular weight and low viscosity for use as an injectable contrast agent for MR imaging. The primary bonds of HMWHA are broken by two main processes used in the current manufacturing of LMWHA: physical methods (such as heat treatment, gamma rays, ozone, electron beams, and ultra-sonication) and chemical procedures (such as enzymatic and acid degradation) (Hokputsa et al., 2003; Choi et al., 2010; Chen et al., 2019).

Among these techniques, γ-ray irradiation demonstrates Newtonian liquid behavior and notably decreases the dynamic viscosity of generated LMWHA when subjected to an applied share rate (Zhang et al., 2014; Huang et al., 2019). This greatly increases the viability of LMWHA as an injectable tumor-targeting therapy.

Conclusions

The synthesis of LMWHA was achieved through the utilization of gamma ray technique in this work. Scientists performed a comprehensive examination of biological and characteristics of LMWHA-Fe_3O_4 nanoparticles after merging superparamagnetic Fe_3O_4 nanoparticles with LMWHA. Various techniques were employed, such as superconducting quantum interference devices, electrophoretic light scattering, X-ray diffraction (XRD) and viscosity testing. Additionally, the capacity of LMWHA-Fe_3O_4 NPs to specifically target MCF7 breast cancer cells was assessed through the use of the thiocynate method and time-of-flight secondary ion mass spectrometry (TOF-SIMS).

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