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Laura Metsämäki

HYDROGELS AS CANCER THERANOSTIC PLATFORMS

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ABSTRACT

Laura Metsämäki: Hydrogels as cancer theranostic platforms

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Theranostic hydrogels combine cancer therapy and diagnostics on a single platform. Hydrogels are water-absorbing polymer networks that can embed drugs and imaging agents inside their structure. Stimuli-responsive hydrogels can release the embedded agents in response to stimuli. For example, pH, temperature, ultrasound, light, and magnetic field can initiate the swelling of stimuli-responsive hydrogels and thus induce the release of different agents. The structural changes of Schiff base bonds or ionizable groups give rise to the swelling of pH-sensitive hydrogels. Since the tumor environment is acidic, pH-sensitive hydrogels are often utilized in cancer treatment. Temperature-sensitive hydrogels are based on polymers that can transform from solution state to gel state in response to temperature variation. As for the ultrasound, it can inflict the release of drugs and imaging agents indirectly by producing an increase in temperature and pressure. Light sensitivity can be achieved by adding functional groups to the polymer network that can go through cis-trans-isomerization or other structural changes in response to light. While magnetic particles inside the hydrogel cause sensitivity to the magnetic field.

Hydrogels can be delivered to the body environment in different ways. The injection of hydrogels is one of the most common delivery strategies since it is minimally invasive and enables the transportation of the hydrogel to various body parts. In order to have a successful injection, hydrogels must have suitable properties for gelling. In addition to injections, hydrogels can be delivered orally to treat gastric and colon cancer. Orally delivered hydrogels must be durable because they have to endure the enzymatic and acidic conditions of the digestive tract. Pulmonary delivery enables the treatment of lung cancer via inhalation of the hydrogel. Whereas the nose cavity provides a great position for brain cancer treatment. Similar to the digestive tract, also respiratory tract is covered by mucous membrane, and therefore the bioadhesive properties of hydrogels are especially important. In the case of melanoma treatment, hydrogel-based microneedle patches have shown excellent results. Hydrogels can be also surgically implanted but due to the invasiveness of the operation, hydrogel implantation is usually only utilized when it is combined with compulsory surgery.

Theranostic hydrogels are embedded with imaging agents. For some diagnostic techniques, the imaging agents are a necessity, whereas for some techniques their task is only to improve the accuracy by enhancing the signals. Imaging agents can accumulate in tumors, or they can remain in the hydrogel and thus provide information on the hydrogel degradation. In addition to hydrogel degradation, theranostic hydrogels can also gather information on drug distribution, cancer progression, tumor size, and effectiveness of the treatment. Diagnostic imaging can be performed for example by magnetic resonance imaging and optical imaging. This work intends to offer information on theranostic hydrogels in cancer treatment and introduce the reader to the essential aspects that need to be considered in their development.

Keywords: Theranostic hydrogels, cancer treatment and diagnostics, stimuli-responsive hydrogels, hydrogel delivery

TIIVISTELMÄ

Laura Metsämäki: Hydrogeelit syövän hoidon teranostisina alustoina

Kandidaatintutkielma

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Teranostiset hydrogeelit yhdistävät syövän hoidon ja diagnostiikan samalle alustalle. Hydrogeelit ovat vettä sitovia polymeeriverkkoja, joiden sisään voidaan sulkea lääkkeitä ja kuvantamisaineita. Älykkäät hydrogeelit pystyvät vapauttamaan sisäänsä suljetut aineet ärsykkeelle altistuneena. Ne turpoavat ja siten vapauttavat sisältönsä esimerkiksi pH:n, lämpötilan, ultraäänen, valon, tai magneettikentän stimuloimana. pH-herkkien hydrogeelien rakenteesta löytyy usein Schiffin emäksiä tai ionisoitavia ryhmiä, joiden rakenteelliset muutokset aiheuttavat hydrogeelin turpoamisen pH:n vaihdellessa. pH-herkkiä hydrogeelejä käytetään usein syövän hoidossa ja diagnostiikassa, koska kasvaimen ympäristö on hapan. Sen sijaan lämpötilalle herkät hydrogeelit hyödyntävät polymeerejä, jotka geeliytyvät tai muuttuvat liuosmaiseen muotoon lämpötilan muuttuessa. Puolestaan ultraääni pystyy vapauttamaan lääkkeet ja kuvantamisaineet hydrogeelin sisältä välillisesti lämpötilannousun ja paineen vaihtelun avulla. Valolle herkät ryhmät pystyvät aiheuttamaan muutoksia hydrogeelin rakenteeseen esimerkiksi cis-trans-isomeroitumisella. Sen sijaan, magneettiset partikkelit hydrogeelissä aiheuttavat herkkyyden magneettikentälle.

Hydrogeelejä pystytään kuljettamaan kehoon monilla eri tavoin. Hydrogeelin injektointi on yksi yleisimmistä kuljetusmenetelmistä, sillä se on nopea, minimaalisesti invasiivinen ja sen avulla pystytään kuljettamaan hydrogeelejä monipuolisesti eri puolille kehoa. Hydrogeelien geeliytymiskäyttäytymisellä on suuri rooli injektoimisen onnistumiseen. Injektoinnin lisäksi hydrogeelejä voidaan kuljettaa oraalisesti paksusuolen- sekä mahasyövän hoitoa varten. Oraalisesti kuljetettavien hydrogeelien tulee olla kestäviä, sillä olosuhteet ruuansulatuskanavassa ovat happamat ja entsymaattiset. Keuhkosyövän hoitoa varten on tutkittu myös hydrogeelejä, jotka pääsevät keuhkoihin hengityksen avulla. Nenäontelo sen sijaan tarjoaa paikan hydrogeeleille aivokasvainten hoitoa varten. Ruuansulatuskanavan tapaan myös hengitysteiden pinnat on peitetty limakalvolla ja siksi hydrogeelien adhesiiviset ominaisuudet ovat erityisen tärkeitä. Ihosyövän hoidossa voidaan käyttää hydrogeelejä, jotka hyödyntävät mikroneulausta. Hydrogeelejä pystytään kuljettamaan myös kirurgisesti leikkaamalla, mutta johtuen toimenpiteen invasiivisuudesta, kyseistä tapaa on järkevintä käyttää vain pakollisten leikkauksien yhteydessä.

Teranostisten hydrogeelien sisään upotetaan kuvantamisaineita. Joillekin kuvantamistekniikoille kuvantamisaineet voivat olla välttämättömyys, kun taas toisille ne vain parantavat tulosten tarkkuutta vahvistamalla signaaleja. Kuvantamisaineet voivat esimerkiksi kerääntyä kasvaimen ympärille tai ne voivat myös jäädä hydrogeelin sisälle antamaan tietoa hydrogeelin hajoamisesta. Hydrogeelien hajoamisen lisäksi teranostisten hydrogeelien avulla voidaan tutkia vapautuneiden lääkeaineiden levittäytymistä, syövän etenemistä, hoidon tehokkuutta ja kasvainten kokoa. Kuvantamista voidaan tehdä monien menetelmien kuten magneettiresonanssikuvauksen sekä optisen kuvantamisen avulla. Tämän työn tarkoituksena on tarjota tietoa ternaostisista hydrogeeleistä syövän hoidossa sekä esitellä niiden suunnittelussa huomioitavia näkökulmia.

Avainsanat: Teranostiset hydrogeelit, syövän hoito ja diagnostiikka, älykkäät hydrogeelit, hydrogeelien kuljetus

PREFACE

This work is a Bachelor's thesis done for Tampere University. I would like to thank my supervisor Christine Gering for providing valuable guidance and excellent ideas throughout the whole writing process.

Tampere, 26th April 2022

Laura Metsämäki

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

^{188}Re	Rhenium-188
Ag_2S	Silver sulfide
CEC	N-carboxyethyl chitosan
CT	X-ray computed tomography
Fe_3O_4	Iron(II,III)
$FeCl_3$	Iron(III) chloride
HA	Hyaluronic acid
LCST	Low critical solution temperature
miRNA-210	MicroRNA-210
MRI	Magnetic resonance imaging
$Na^{188}ReO_4$	Sodium Perrhenate
NIR	Near-infrared
PA	Photoacoustic imaging
PAA	Poly(acrylic acid)
PEDGA	Dibenzaldehyde-terminated poly(ethylene glycol)
PEG	Polyethylene glycol
PET	Positron emission tomography
PNIPAM	Poly(N-isopropylacrylamide)
SPECT	Single-photon emission computed tomography
UCST	Upper critical solution temperature

1. INTRODUCTION

Cancer, the leading cause of death worldwide, refers to a disease in which malignant cells grow abnormally fast and spread throughout the body. There are various cancer treatment options, and they include for example surgery, chemotherapy, radiation therapy, immunotherapy, photothermal therapy, and photodynamic therapy. However, many of these treatment options are inefficient, invasive, lack precise targeting, and cause diverse side effects. The utilization of hydrogels can remediate the difficulties in question. (Z. Sun et al., 2020; F. Wang et al., 2021)

Hydrogels are hydrophilic polymer networks that can absorb large amounts of water. Hydrogels can be used in many applications such as tissue engineering scaffolds (Lam et al., 2014), wound dressings (Yu et al., 2021), contact lenses (Talu, 2021), diagnostic platforms (Jaiswal et al., 2014), and drug delivery systems (Sharpe et al., 2014). Hydrogels that combine diagnostics and therapy on a single platform have drawn much attention lately in cancer treatment and they can be referred to as hydrogel theranostic platforms (F. Wang et al., 2021).

Hydrogel theranostic platforms incorporate drugs and imaging agents inside their three-dimensional mesh structure. Swelling hydrogel gradually releases the embedded substances, and subsequently, enables the diagnosis and the treatment of the disease. Essential therapeutic agents for example in chemo- and radiotherapy can be embedded inside the hydrogel and released in a controlled manner. The hydrogel-assisted controlled release offers long-term, well-targeted, and continuous treatment and diagnosis compared to free agents. The hydrogel also protects the agents and prevents them from degrading. Hydrogel theranostic platforms offer personalized treatment for patients, as the treatment can readily be altered according to diagnostic results. One of the main advantages of theranostic platforms is that the imaging agents assist in evaluating the efficacy of the therapeutic agents. (Shetty et al., 2019; F. Wang et al., 2021)

This thesis aims to introduce the principal concepts for the design of hydrogel theranostic platforms for cancer treatment. The intention is also to provide necessary information for a competent comparison between methods and mechanisms. In the first chapter of the thesis different drug release mechanisms and typical hydrogels for each release mechanism are presented. The second chapter deals with the different delivery strategies of theranostic platforms, and the third chapter discusses the various diagnostic approaches. In the final chapter, a hypothetical theranostic platform in cancer treatment is introduced.

2. HYDROGELS AND THEIR RELEASE MECHANISMS IN CANCER THERAPY

Hydrogels can carry drugs and imaging agents inside of their network structure. Smart/stimuli-responsive hydrogels can change their physiochemical properties and release the drugs and imaging agents in response to stimuli. (Wei et al., 2021) Hydrogels are relatively soft materials, and they are easily subjected to mechanical stimuli (W. Sun et al., 2018). Under a certain stimulus, the interactions between polymers and polymer-solvents change, and the hydrogel starts to degrade or change its conformation. Consequently, the drugs and imaging agents are being released. (Z. Sun et al., 2020) The macroscopical changes of stimuli-responsive hydrogels are reversible. Meaning that, when the stimulus ends, they can recover to their initial stage. (Bustamante-Torres et al., 2021) Besides the stimuli-responsive hydrogels, also diffusion-based drug release can be utilized (F. Wang et al., 2021). In this thesis, the focus is on stimuli-responsive hydrogels.

The different types of stimuli can be categorized into three groups: physical, chemical, and biochemical stimuli. Light and temperature are great examples of physical stimuli. Whereas enzymes and antigens are good examples of biochemical stimuli. On the other hand, exposure to changing pH and oxidants affect the behavior of chemically responsive hydrogels. (Sahle et al., 2018; Ullah et al., 2015) The drug release mechanism is dependent on the polymer types and potential substances that are incorporated into the hydrogel structure (F. Wang et al., 2021).

Dual- and triple-sensitive hydrogels can react to multiple stimuli simultaneously. For instance, pH-, magnetic- and thermosensitive hydrogels are fabricated by copolymerizing temperature- and pH-sensitive monomers and incorporating magnetic particles among them. (Hayati et al., 2020; Yue et al., 2019) Hydrogels with multiple drug release mechanisms often offer more precise drug delivery and they adapt easily to different physiological environments (Chatterjee & Hui, 2021).

This chapter handles different mechanisms of stimuli-responsive hydrogels. While this thesis does not aim to provide a comprehensive review, various polymers used for specific release mechanisms and relevant literature examples are introduced. In this chapter, hydrogels sensitive to pH, temperature, light, ultrasound, and magnetic field are examined. Some stimuli-responsive hydrogels, that have been under research, are presented in Table 1. Besides their release mechanisms, also their origins, incorporated drugs, and applications are introduced.

Table 1: Examples of stimuli-responsive hydrogels

Hydrogel	**Stimulus sensitivity**	**Origin**	**Active substance/ Drug**	**Application**	**References**
Pluronic/Poly(acrylic acid)	pH, temperature	Synthetic	Epirubicin	Colorectal cancer therapy	(Lo et al., 2013)
N-carboxyethyl chitosan/ Poly(ethylene glycol)	pH	Hybrid	Doxorubicin	Hepatocellular carcinoma therapy	(J. Qu et al., 2017)
Alignate/Chitosan	pH	Natural	Resveratrol	Cancer therapy	(Nazli & Acikel, 2019)
Poly(*N*-isopropylacrylamide-*co*-itaconic acid)/Chitosan	pH, temperature	Hybrid	Doxorubicin	Breast cancer therapy	(Fathi et al., 2019)
Synthetic Peptide (sequence FEFEFRFK)	pH	Natural	Paclitaxel	Cancer therapy	(Raza et al., 2019)
Poly(organophosphazene)	Temperature	Synthetic	2-Methoxyestradiol	Breast cancer therapy	(Cho et al., 2011)
Chitosan	Temperature	Natural	Disulfiram	Cancer therapy	(Ahsan et al., 2020)
Dopamine-functionalized hyaluronic acid	Ultrasound, temperature (Gold nanoparticles)	Natural	-	Cancer therapy	(An et al., 2021)
Poly(ethylene glycol)/ Poly(lactic-co-glycolic ac-	Ultrasound, temperature	Synthetic	Doxorubicin	Cancer therapy	(C.-H. Wu et al., 2020)

id)/ 2,2′-Bis (2-oxazoline)					
Chitin	Ultrasound, temperature	Natural	Gallic acid	Cancer therapy	(Jiang & Kobayashi, 2017)
Alginate	Light (Indocyanine green)	Natural	Doxorubicin	Cancer therapy	(Anugrah et al., 2019)
Poly(*N*-acryloyl glycinamide-*co*-acrylamide)	Light, temperature (Polydopamine coated-gold nanoparticles)	Synthetic	Doxorubicin	Breast cancer theranostics	(Y. Wu et al., 2018)
α-cyclodextrin modified glucan curdlan/ azobenzene modified poly(acrylic acid)	Light	Synthetic	-	Drug delivery	(Tamesue et al., 2010)
Alignate/Gelatin	Magnetic field (Fe_3O_4-nanoparticles)	Natural	Doxorubicin	Cancer therapy	(Jahanban-Esfahlan et al., 2020)
Chitosan	Magnetic field, (Fe_3O_4-nanoparticles)	Natural	Substances of bacillus Calmette–Guérin	Bladder cancer therapy	(D. Zhang et al., 2013)
Poly(*N*-isopropylacrylamide)	Magnetic field, pH, temperature, (Fe_3O_4-nanoparticles)	Synthetic	Doxorubicin	Cancer therapy	(Davaran et al., 2014)
Chitosan/Telechelic difunctional poly(ethylene glycol)	Magnetic field, temperature, (Fe_3O_4-nanoparticles)	Hybrid	Doxorubicin, docetaxel	Breast cancer therapy	(Xie et al., 2017)

2.1 pH-sensitive hydrogels

pH-sensitive hydrogels are excellent drug carriers especially in cancer treatment. The drug release of pH-sensitive hydrogels is based on the pH difference of the hydrogel surrounding. In the tumor microenvironment, the pH can range from 5,8 to 7,2, whereas the pH of normal tissue is approximately 7,4. (Z. Sun et al., 2020) The pH is lower in the tumor microenvironment because cancer cells have a higher metabolic rate than normal cells. In particular, their glucose uptake is larger and there is poor perfusion in the tumor environment. Consequently, it is not possible to remove the increasing amounts of metabolic waste and the environment grows more acidic. (X. Zhang et al., 2010) There are two predominant chemical structures that enable pH sensitivity: Schiff base bonds, and ionizable groups (F. Wang et al., 2021).

Schiff base bonds are covalent dynamic bonds that can degrade in acidic conditions (F. Wang et al., 2021; Zhou et al., 2014). They act as molecular switches that are dependent on pH (Xu et al., 2019). When the Schiff base bonds degrade, the crosslinking density changes and the hydrogel starts to swell (Zhou et al., 2014). Schiff base bonds also offer a way for self-healing based on the pH change within the system. When the pH is reverted to a neutral regime, the Schiff-base pairs will form a covalent bond again, thus offering a self-healing hydrogel. (Guo et al., 2019)

Ionizable groups are more commonly used to achieve pH sensitivity compared to Schiff base linkages (F. Wang et al., 2021). Here, the hydrogel properties change based on ionic/functional groups donating or accepting protons. In cancer treatment, cationic hydrogels are often utilized because they can release the drug in acidic conditions. As mentioned earlier, the tumor microenvironment is acidic and hence suitable for cationic hydrogels. On the other hand, anionic hydrogels release the drug in an alkaline environment and therefore are usually not suitable for releasing drugs in the tumor microenvironment. (Andrade et al., 2021; Ullah et al., 2015) Cationic hydrogels often contain amino or imino groups. When these groups are protonated, hydrogels swell because positively charged moieties repel each other. Contrarily, chain repulsion can occur when the acidic groups of anionic hydrogels, such as carboxylic or sulfonic acids, are ionized. (Andrade et al., 2021; Ullah et al., 2015)

Natural and cationic polymer chitosan and its different derivatives are often incorporated into the structure of pH-sensitive hydrogels (Andrade et al., 2021). In one study, a hydrogel composed of N-carboxyethyl chitosan (CEC) and dibenzaldehyde-terminated poly(ethylene glycol) (PEDGA) was loaded with doxorubicin. The hydrogel utilized Schiff base linkages in drug release for hepatocellular carcinoma therapy. (J. Qu et al., 2017) In addition, synthetic poly(acrylic acid) (PAA) is often used to make pH-sensitive hydrogels. A large number of acidic groups in its structure enables its pH sensitivity. (Rizwan et al., 2017) Due to the poor mechanical and thermal properties of

PAA, hydrogels that are based on PAA are often reinforced with other polymers (Lim et al., 2015).

2.2 Thermosensitive hydrogels

Temperature change is almost inevitable when delivering hydrogels into the body, and therefore thermosensitive hydrogels are one of the most common stimuli-responsive hydrogels. Typically, thermosensitive hydrogels release drugs when temperature increases, and this mechanism is usually based on their low critical solution temperature (LCST). Above LCST, hydrogels are in a gel state and under LCST they are in a solution state. (Z. Sun et al., 2020) The transition between the solution and gel state can be called sol-gel transition or positive response and appears as swelling of the hydrogel. In the gel state, the polymers have hydrophobic behavior and in the solution state, they are in a hydrated condition. Hydrogels are often delivered to the body via injection which requires them to be in a solution state. Whereas, when they are in the body they should optimally be in the gel state. Therefore, the ideal LCST for hydrogel drug delivery is between room temperature and body temperature. (Bustamante-Torres et al., 2021) Due to sol-gel transition, the polymeric network transforms into a well-organized matrix (Rafael et al., 2021). The reaction is caused by the polymer composition. Thermosensitive hydrogels contain both hydrophilic and hydrophobic portions. Temperature change affects the interactions, such as hydrogen bonds, between the polymers and the water molecules and can be seen as swelling. (Y. Zhang et al., 2016)

Furthermore, hydrogels can also have upper critical solution temperature (UCST) in which they transition from gel to sol. This reaction can be also called a negative temperature response. It can be seen as the shrinking of the hydrogel when temperature increases. (Bustamante-Torres et al., 2021) For instance, polyethylene glycol (PEG) behaves like this in an aqueous environment (Meenach et al., 2010). However, UCST- exhibiting polymers are not as common as LCST- exhibiting polymers (Ehrenhofer et al., 2018).

Poly(N-isopropylacrylamide) (PNIPAM) is a commonly used thermosensitive synthetic polymer (Hayati et al., 2020). Its LCST is 32 °C, which is close to body temperature and thus ideal for drug delivery. Copolymerization of PNIPAM with other polymers is a standard process that can be used to alter its LCST, biodegradability, and mechanical properties. (Klouda & Mikos, 2008; Z. Sun et al., 2020)

2.3 Ultrasound-sensitive hydrogels

Ultrasound is an acoustic wave that has a frequency above 20 kHz (Fateh et al., 2021). Ultrasound is a multipurpose tool when it comes to cancer theranostic platforms. It can be used in the diagnosis of cancer but also it can be used to induce drug release. Ultrasound has a good penetration ability, it is non-invasive, and the exposure can be limited to a certain area with focusing techniques. Compared to ultraviolet and visible light, ultrasound is capable to penetrate deeply into the living tissue. (Yamaguchi et al., 2019) In addition, ultrasound does not damage the hydrogel structure permanently (Wei et al., 2021).

Ultrasound can affect living tissue in two ways: thermally and nonthermally. Thermal effects can be seen as a rise in temperature in the irradiated tissue. Consequently, it gives rise to perturbation of the cell membrane and increased permeability of the blood vasculature. On the other hand, non-thermal effects are mostly based on the cavitation effect. Cavitation is a phenomenon where micro- or nanobubbles are generated due to a pressure drop. The bubble formation increases the pressure, enhances vascular permeability, and advances intracellular drug uptake. The thermal and non-thermal effects cause the destabilization of the hydrogel system and thus promote drug release. (Z. Sun et al., 2020; Wei et al., 2021; A. Zhang et al., 2019)

Utilizing ultrasound in clinical applications has promising and broad prospects. In addition to drug release, ultrasound has also other advantages related to drug therapy. For example, it can be used to disrupt the blood-brain barrier and thus advance the entry of therapeutic molecules into the brain. Also, it can be used to deliver therapeutic agents through the skin. However, ultrasound is not a completely harmless tool, because it may induce DNA damage and thus lead to cancer. (Wei et al., 2021) In addition, thermal injury of tissue, free radical generation, and drug degradation may be a problem (Fateh et al., 2021; Kubota et al., 2021).

Various polymers and structures have been exploited to create ultrasound-sensitive hydrogels. Certain basic principles must be taken into account when designing an ultrasound-responsive hydrogel. Firstly, the structure of the hydrogels must be somewhat stable to prevent drug leaking. However, the structure cannot be too rigid because it still needs to respond to mechanical stimuli. This kind of structure can be achieved for example by incorporating permanent and dynamic crosslinkers. In one study, researchers achieved this goal by permanently crosslinking methacrylated hyaluronic acid (HA) and polyethlylene-glycol acrylate, while the therapeutic agent, tannic acid, was dynamically crosslinked to the hydrogel backbone. The dynamic bond facilitates the release of tannic acid. (W. Sun et al., 2018) Secondly, the porous morphology of the hydrogel is essential when wanting to enable cavitation, because cavitation bubbles collapse in the micropores (P. Zhang et al., 2020). Due to the thermal effects of ultrasound, polymers that are used to fabricate thermosensitive hydrogels are also a popular choice for ultrasound-sensitive hydro-

gels. For example, a thermosensitive PNIPAM can be used in this application as well. (C.-H. Wu et al., 2018)

2.4 Photosensitive hydrogels

Light induces drug release in a safe and controlled manner. Its properties such as wavelength and intensity are easy to adjust (L. Li et al., 2019; Yang et al., 2020). Light is a versatile tool because it can induce hydrogel degradation or formation. It has the ability to initiate network contraction or expansion or it may create chemical modifications to the network. Photosensitivity is achieved by incorporating photo-responsive groups into a hydrogel, which can be located in the crosslinking points, along the polymer backbone, or along the side chains. Alternatively, photo-responsive substances can be added to the aqueous hydrogel formulation, which will indirectly change the hydrogel properties upon activation through host-guest interactions. The effects of the light can be usually seen as hydrogel swelling and shrinking. (L. Li et al., 2019)

Azobenzenes are the most studied and effective units that can be used to achieve photosensitivity in hydrogels. Azobenzenes can be incorporated into the drug delivery system by adding an azobenzene-containing surfactant. Azobenzenes take either *cis* or *trans* configuration depending on the wavelength of the light as can be seen in Figure 1. The *trans* isomer is more hydrophobic whereas the *cis* isomer is more hydrophilic. Therefore, when azobenzenes are in the *cis* form, the hydrogel starts to swell. On the other hand, when azobenzenes are in the *trans* form surfactants bind stronger to the microgel which leads to hydrogel contraction. After ultraviolet light of around 365 nm is induced, almost all azobenzenes are in the *cis* configuration. If the light is changed back to visible light and it can take two days for the azobenzenes to change back to the *trans* configuration. Especially hydrogels that form crosslinks between azobenzenes and cyclodextrins have raised great interest. Cyclodextrin is a cyclic oligosaccharide compound, that can form host-guest complexes with azobenzenes when the azobenzenes are in the *trans* configuration as can be observed in Figure 1. (L. Li et al., 2019; Yang et al., 2020; Zakrevskyy et al., 2012) Tamesue *et al.* (2010) developed a photo-switchable hydrogel by utilizing cyclodextrins and azobenzenes. The hydrogel was composed of host polymer curdlan that contained also cyclodextrin and guest polymer PAA that was modified with azobenzene. Photoirradiation successfully induced the isomerization of azobenzene to *cis* configuration and thus dissociation between the two complexes.

The use of photosensitive hydrogels is not completely free of problems. For instance, the overheating of surrounding tissue remains unaddressed and hydrogels with sensitivity to low-energy light have not yet been developed. (L. Li et al., 2019) Additionally, compared to for example ultrasound, the penetration depth of light into living tissue is limited (Yamaguchi et al., 2019).

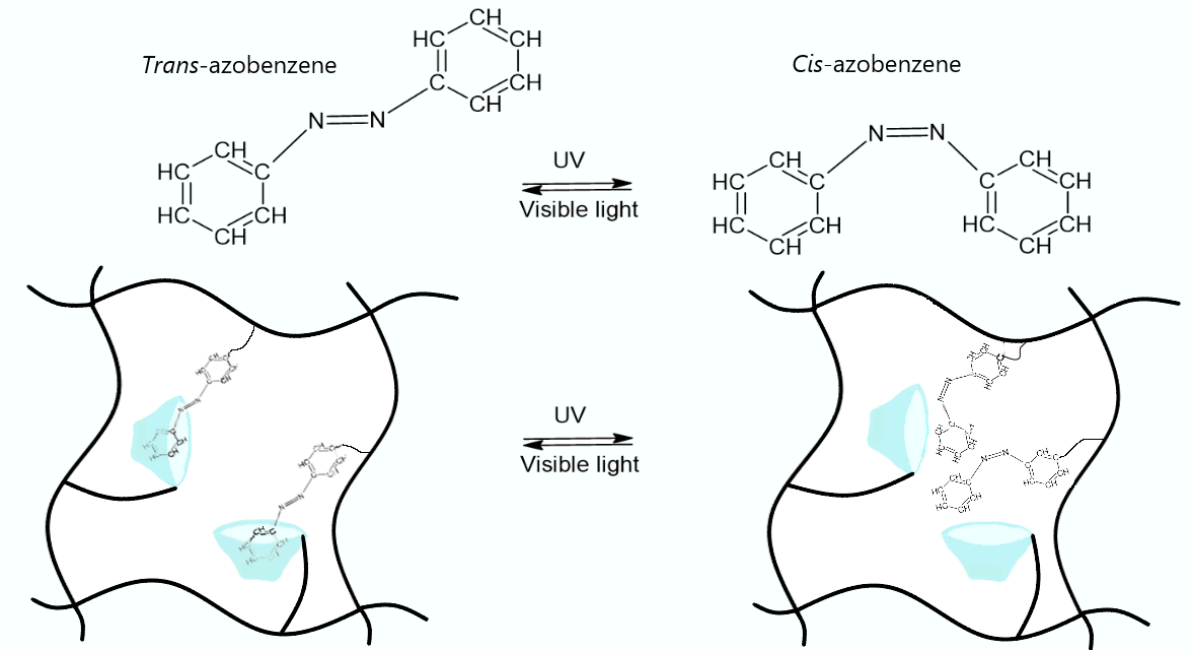

Figure 1: The image demonstrates the light-induced cis-trans isomerization of azobenzenes. Hydrogel swelling can be controlled by crosslinking azobenzenes and cyclodextrins (in blue) into the polymer network. The light-dependent host-guest relationship between trans azobenzenes and cyclodextrins is presented. The illustration is inspired by (C. Wang et al., 2018)

2.5 Magnetic-sensitive hydrogels

The use of the magnetic field in hydrogel drug release is quick, non-invasive, and temporary. The sensitivity to magnetic field originates from magnetic-responsive additives, that are combined with the hydrogel network. Common additives are iron oxides, transition metal ferrites, and transition metal alloys. (Z. Li et al., 2021) Iron oxides such as Fe_3O_4 are the most common magnetic nanoparticles used to achieve stimuli responsiveness (Liu et al., 2020).

Magnetic nanoparticles vibrate under magnetic field exposure. The vibration of the magnetic-responsive additives induces swelling of the hydrogel. When the hydrogel is not stimulated by a magnetic field, polymers in the hydrogel network often form interactions with magnetic-responsive additives. Due to the interactions, the network is dense. Contrarily, when magnetic-responsive additives vibrate, the interactions fade, and the hydrogel starts to swell. It has been shown that the swelling ratio is dependent on the number of magnetic particles in the hydrogel. (F. Lin et al., 2019) In addition, the vibration increases the temperature which may further affect drug release. The increased temperature enhances the movement of drug molecules and thus intensifies drug diffusion. Moreover, the drugs are released more efficiently because polymers dissolve and de-

grade under heat. (Z. Li et al., 2021) Due to this thermal effect, thermosensitive polymers are often utilized with magnetic-responsive additives (Andrade et al., 2021).

As mentioned, the key for fabricating magnetic-sensitive hydrogels is to incorporate magnetic-responsive additives into the hydrogel. Therefore, a wide range of different polymers is used to produce magnetic-sensitive hydrogels. Natural polymers, such as collagen and chitosan, are popular choices for making magnetic-sensitive hydrogels. For instance, D. Zhang *et al.* (2013) developed chitosan hydrogel with Fe_3O_4 nanoparticles for bladder cancer treatment. The hydrogel was sensitive to temperature change and magnetic field. In addition, a thermoresponsive magnetic hydrogel was developed by Jaiswal *et al.* (2014) for cancer theranostics. The hydrogel was based on PNIPAM and it utilized Fe_3O_4 as magnetic nanoparticles. Radiofrequency exposure successfully induced the release of doxorubicin. (Jaiswal et al., 2014)

3. DELIVERY STRATEGIES FOR CANCER THERANOSTIC HYDROGELS

Theranostic platforms can be delivered to the body environment in different ways. In this chapter, injection, oral delivery, pulmonary delivery, nasal delivery, microneedle patch, and surgical implantation are the presented delivery strategies. Multiple aspects must be considered before choosing the right delivery method.

The location of cancer has a major impact on the selection of a drug delivery strategy. It is evident, that tumors that are located beneath multiple tissue layers require a different kind of delivery strategy than those that occur in the uppermost tissue layers. For example, to treat glioma, multiple tissue layers of the skull have to be pierced in order to reach the tumor. Whereas in the case of melanoma, hydrogels only must penetrate through superficial skin layers.

Another aspect that affects the drug delivery strategy, is the size of the hydrogel. The transport in the blood vessels, airways, and gastrointestinal tracts happens differently depending on the size of the hydrogel. According to their size, hydrogels can be roughly categorized into three groups: macroscopic gels, microgels, and nanogels. The size of the macroscopic gels varies from millimeters to centimeters. They can be injected or directly implanted. Even though macroscopic hydrogels can’t penetrate epithelial barriers, they still can be used in transdermal drug delivery. On the other hand, the size of the microgels varies from 0.5μm to 10μm, whereas nanogels are smaller than 200nm. Microgels are often used in oral and pulmonary delivery and nanogels are best suited for the systemic administration of drugs. The superficial area of micro-and nanogels is larger than macroscopic hydrogels which improves penetration into tissue but also increases the clearance rate, phagocytosis by macrophages, and covalent bonding with other molecules. (J. Li & Mooney, 2016; Z. Sun et al., 2020)

In order to treat cancer at a specific site, hydrogels need to adhere to the tissue surface. Thus, the bioadhesive properties of a hydrogel have to be suitable for a specific drug delivery strategy. Hydrogels that are delivered to the body via oral administration, confront different conditions than hydrogels that are utilized on the skin surface. Orally administrated hydrogels need to adhere to the wet surface of the intestinal epithelium, hence they require excellent adhesive properties. In addition, hydrogel needs to be tough enough to avoid rupture during drug delivery. Some strategies require the hydrogel to withstand greater forces than others. (J. Li & Mooney, 2016)

Furthermore, some of the strategies are preferred over others due to safety reasons. Typically, the aim is to avoid invasive methods because a surgery leads to a bigger risk and patient discomfort. Therefore, injectable hydrogels have been preferred over hydrogels that require surgical implantation.

3.1 Injection

Injected hydrogels are transferred into the body via a syringe or catheter (Z. Sun et al., 2020). Hydrogel injection is a common delivery strategy, because it is rapid, does not cause scarring, can be used to reach tumors beneath multiple tissue layers, and has only a small risk for infection (Kasiński et al., 2020; F. Wang et al., 2021). A wide variety of cancer types can be treated and diagnosed with injectable hydrogels. As mentioned, an injection needle can reach tumors that are beneath multiple tissue layers. For example, there has been research for injectable hydrogels in the treatment of breast cancer (Fathi et al., 2019), renal cell carcinoma (Ueda et al., 2016), lung cancer (N. Wang et al., 2021), gastric cancer (Gangrade & Mandal, 2020), pancreatic cancer (Phan et al., 2016), melanoma (J. Liu et al., 2016) and brain cancer (de la Puente et al., 2018). Compared to many other delivery methods, injectable hydrogels can be delivered directly into the tumor or alongside it (Fan et al., 2019).

When the injection is used as a drug delivery strategy, the hydrogel size must be carefully considered. The location of the injection affects the requirements for suitable gel size as well. An intravascular injection is not suitable for microgels that are smaller than 5 µm, because they will be removed from the blood plasma too rapidly. On the other hand, nanogels can be used in this application when their size is between 10 and 100 nm. The size in question allows the movement of the hydrogel from blood vessels to the target tissue but does not allow clearance by the kidney or phagocytizing by macrophages, which are problems for hydrogels that are even smaller. (J. Li & Mooney, 2016) Compared to for example surgically implanted hydrogels, injectable hydrogels need to be in a solution-like state, because the hydrogel must be extruded through the syringe. There are two gelation types that injectable hydrogels utilize: *in situ* gelation and shear-thinning. The gelation strategy is dependent on the interactions between the polymers. Chemically crosslinked hydrogels utilize *in situ* gelation whereas physically crosslinked hydrogels take advantage of shear-thinning. (F. Wang et al., 2021)

As the name indicates, *in situ* gelling hydrogels go through sol-gel transition inside of the body (J. Li & Mooney, 2016). Covalent bonds are formed after injection by polymerization or self-assembly. In the case of polymerization, usually a stimulus such as light initiates the formation of non-reversible covalent bonds within the hydrogel. (Loebel et al., 2017) In the case of self-assembly, the hydrogel goes through reversible or non-reversible reactions that can be started

spontaneously or after certain biological stimuli such as temperature (van Tomme et al., 2008). Click chemistry reactions are often utilized with self-assembling *in situ* gelling hydrogels. Click chemistry refers to a group of reactions that combine various units. Schiff reactions are typical examples of click chemistry. (J. Xu et al., 2019) *In situ* gelling hydrogels are often slow-gelling systems. This is favorable because the gelation can be started before the hydrogel enters the body, but the system is still in a solution-like state to flow through the tip of the syringe. In addition, it prevents dilution of the hydrogel upon entering the target site. (J. Li & Mooney, 2016) Thermosensitive polymers are a practical choice for making *in situ* gelling hydrogels. As told, polymers that possess LCST can change from liquid to gel when the temperature is increasing. Thereby, PNIPAM is a popular polymer for injectable hydrogels. (Ekenseair et al., 2012)

Conversely, shear-thinning hydrogels undergo a sol-gel transition before they are injected. In other words, the gelling already happens *ex vivo*. However, when hydrogels are injected, shear stress causes viscous flow and thus enables the movement of the hydrogel from the syringe into the body. Shear-thinning hydrogels have reversible physical crosslinks, for example, hydrogen bonds, host-guest interactions, electrostatic interactions, or hydrophobic attractions, throughout their structure. Due to the reversibility of shear-thinning hydrogels, hydrogels return to their initial condition after injection. The event where hydrogels recover after stress removal is called self-healing. Compared to *in situ* forming hydrogels, shear-thinning hydrogels have the advantage of proper characterization of hydrogel features. In addition, shear-thinning hydrogels regain their physical properties in less time after injection and thus do not dilute as easily as *in situ* gelling hydrogels. (Koetting et al., 2015; J. Li & Mooney, 2016; Loebel et al., 2017)

3.2 Oral delivery

Oral delivery is an effective way to transport hydrogels into the gastrointestinal tract (Z. Sun et al., 2020). Hydrogels offer protection for the agents inside the hydrogel structure, as the environment in the gastrointestinal tract is complex and often not ideal for the agents (Sharpe et al., 2014). Oral delivery is non-invasive and has high patient compliance, but the strategy suffers from poor targeting (X. Gao, He, et al., 2013; Javanbakht & Shaabani, 2019; Z. Sun et al., 2020). However, oral delivery has shown great results in the treatment of gastric and colon cancer. Microgels are suitable for oral delivery because their size is large enough to prevent them from entering systemic circulation but small enough to maintain their opportunities for bioconjugation. (Z. Sun et al., 2020)

There are some variables, such as enzymatic and acidic conditions, that need to be taken into account before generating orally administered hydrogels (Gao et al., 2013). In the case of colon cancer treatment, the agents inside of the hydrogel have to be released in almost neutral condi-

tions, because the pH in the colon varies from 7 to 8. Therefore, the hydrogel must endure the low pH values and enzymes in the stomach and the small intestine before it reaches the colon. However, the swelling does not necessarily need to begin until it reaches the colon. In fact, usually the swelling starts gradually after the hydrogel reaches the small intestine. Nevertheless, the aim is to avoid inadvertent targeting and its downsides. (Schoener et al., 2013) Oral delivery to the colon is challenging due to the higher bacterial activity and longer transportation time compared to other gastrointestinal targets (Sharpe et al., 2014). On the other hand, several factors make the colon an effortless target compared to others. For instance, in the colon the residence time is high, there are fewer enzymes and almost neutral pH. (Durán-Lobato et al., 2020) In the case of hydrogels developed for gastric cancer treatment, agents need to be released in very acidic conditions. The pH in the stomach varies from 1 to 3. Naturally, due to this wide variation of pH, pH-sensitive hydrogels are the most typical choice in the treatment of both cancer types. (Schoener et al., 2013)

Mucoadhesion describes the ability of a material to adhere to mucosal membranes. Mucosal membranes can be found for example in the lining of the gastrointestinal and respiratory tract. Generally, hydrophilic polymers that have charged groups or groups that can form hydrogen bonds have good mucoadhesive properties. Mucoadhesion is important because it enables longer residency of a hydrogel at the target site and thus provides better therapeutical results. (Khutoryanskiy, 2011) For instance, one research utilized chitosan that had charged amino groups in its structure so that they would have electrostatic interactions with N-acetylnuraminic acid in gastric mucus (Y.-H. Lin et al., 2015).

3.3 Pulmonary delivery

In pulmonary delivery, hydrogels are transported to the lungs with the help of inhalation. Lung cancer is a typical target to hydrogels that are delivered via the respiratory tract (Z. Sun et al., 2020). Lungs are an attractive target site because they offer a large surface area that has a good blood supply, thin epithelium barriers, and low protease activity (Du et al., 2013; Nikjoo et al., 2021). Pulmonary delivery is a non-invasive and well-targeted technique for cancer treatment and diagnostics. However, some patients may suffer from some side effects such as cough and glossitis. (Ahmad et al., 2020)

Hydrogels that are transported via the respiratory tract have drawn attention due to their suitable size and mucoadhesive properties. Similar to oral delivery, the mucoadhesive properties of hydrogels play an important role because the respiratory tract is covered by the mucosal membrane. After hydrogel inhalation, hydrogels travel to the alveoli, where they adhere to the mucosal membrane. Before they are inhaled, hydrogels are deswelled but after adhesion, they absorb

moisture from the mucosal membrane and swell. This induces the release of the agents inside the hydrogel structure. (Ahmad et al., 2020) In order to achieve the inhalation of hydrogel, aerodynamic performance should be enhanced. This can be made by incorporating dry powder composites with hydrogels. In addition, spray drying is a technique that can be used in changing liquids to dry powders. (Nikjoo et al., 2021; Stocke et al., 2015).

Microgels are often used in pulmonary delivery. The size of the microgels should vary between 1 and 5 µm because bigger hydrogels have the risk of entrapment in respiratory airways whereas smaller hydrogels have the risk of phagocytosis by macrophages. However, there is some research on nanogels used in pulmonary delivery. Nano-sized hydrogels are less likely to be phagocytized but there is a higher chance that they are exhaled out of the body. (Ahmad et al., 2020)

3.4 Nasal delivery

Nasal delivery of hydrogels has shown promising results in treating pathologies of the central nervous system (Bellotti et al., 2021). It is also a potential delivery strategy for brain cancer treatment, and it holds some promise for theranostic use as well (Stawicki et al., 2021; Wen et al., 2011). In nasal delivery, the hydrogel is placed into the nasal cavity, which is positioned between the nostrils and the nasopharynx. The site has a large superficial area but can fit only small volumes. Nasal delivery is non-invasive, and it enables bypassing the blood-brain barrier. However, active substances often lack absorbability when they are administered to the nasal cavity. Also, the location has enzymatic conditions and active mucociliary clearance that affect the longevity of the delivery system. (Lochhead & Thorne, 2012)

One of the most problematic factors in brain cancer treatment is the impermeability of the blood-brain barrier. Tight junctions between endothelial cells of brain capillaries have a major impact on the selectivity of the blood-brain barrier. High selectivity of the blood-brain barrier ensures that toxic agents cannot enter the brain. The blood-brain barrier is however permeable to small lipophilic molecules. Nasal delivery is a superior delivery strategy in brain cancer treatment because the route does not require penetration across blood-brain barriers. (Bruinsmann et al., 2019.) Although also other delivery strategies can bypass the blood-brain barrier, the downside is their invasiveness (Lochhead & Thorne, 2012). The transportation of the active compounds from the nose cavity can happen via the olfactory nerve, trigeminal nerve, lymphatic, vascular pathway, or cerebrospinal fluid (Bruinsmann et al., 2019). In some cases, the active compounds can cross the blood-brain barrier, if they have lipophilic nature and small size (Cardia et al., 2019).

Some studies suggest that hydrogel size in nasal delivery should be between 40 µm and 60 µm since hydrogels smaller than 10 µm have the risk of movement by the airstream and bigger

hydrogels may deposit in the nose. (al Harthi et al., 2019) However, also nano-sized hydrogels are used in nasal delivery. In that case, hydrogels must have good mucoadhesive properties. (Cardia et al., 2019) Similar to oral and pulmonary delivery, the mucoadhesive properties of hydrogels are important because the nasal cavity is lined by the mucous membrane (Robinson et al., 2021).

3.5 Microneedle patch

A microneedle patch is a bandaid-like device that has tiny needles in arrays, delivering a drug transdermally. These microneedles can penetrate the epidermal layer of the skin and transport active molecules to the target site. (Amarnani & Shende, 2021) The micropores enable rapid delivery through multiple tissue layers and therefore increase the bioavailability of active molecules (Amarnani & Shende, 2021). They deliver drugs in a rapid and minimally invasive way producing a minimal amount of pain (M. Qu et al., 2020).

Solid materials such as metal and silicon have been utilized as materials for microneedles. However, solid materials have the risk of leaving residues or fracturing. Therefore, hydrogels are competitive alternatives for this application. Especially hydrogels that have high water content are suitable microneedle patch materials. (Z. Sun et al., 2020) Due to the ability for phase transition, hydrogel-based microneedle patches can penetrate target tissue while being stiff and dry. After they are administered to the target site, they swell, become soft, and release the active molecules. (He et al., 2020) The process in question is illustrated in Figure 2.

In the field of cancer treatment, microneedle patches are mostly used for melanoma treatment. Nevertheless, they can be exploited in the treatment of other cancer types as well. For instance, the use of microneedle patches for pancreatic cancer treatment has been explored. Compared to melanoma treatment, other cancer types require invasive methods for active molecules to reach the tumor site. (Fu et al., 2022) Microneedle patches work well as theranostic platforms. Microneedle patches can extract skin interstitial fluid and can thus be used in biosensing and monitoring physiological parameters. (Amarnani & Shende, 2021; Chew et al., 2020) For instance, microneedle patches can be used in detecting melanoma. Al Sulaiman *et al.* (2019) derived a hydrogel-coated microneedle patch that could detect miRNA-210 from the skin interstitial fluid. miRNA-210 works as a biomarker in the diagnostics of melanoma. The hydrogel coating was composed of alginate polymers that were functionalized with peptide nucleic acids that can sense miRNA-210 from the skin interstitial fluid.

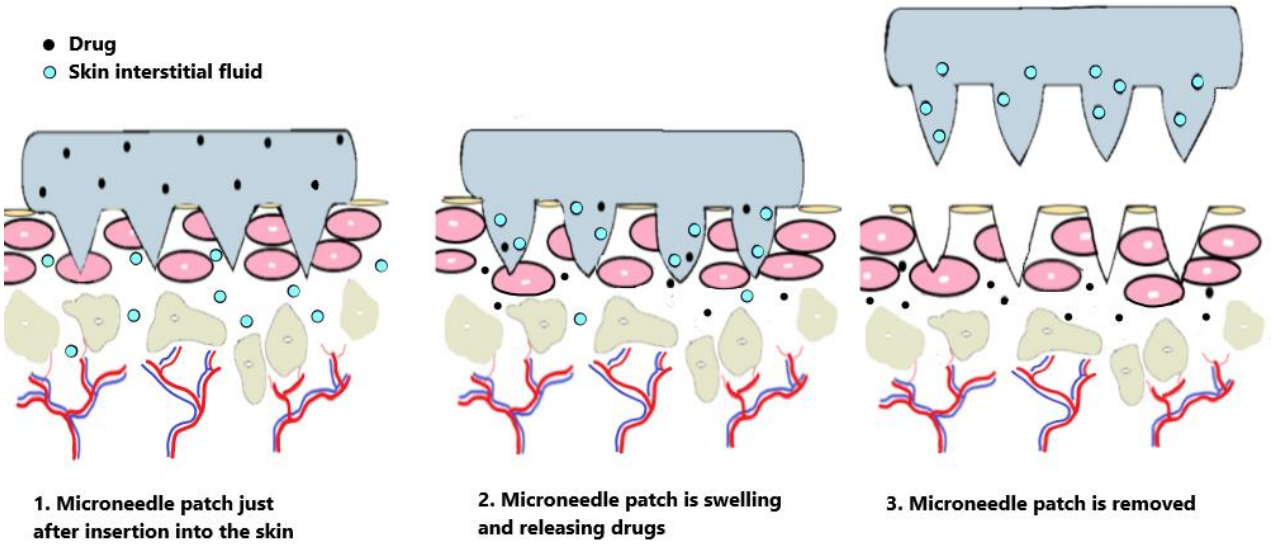

Figure 2: Three stages of hydrogel microneedle patch after insertion into the skin. Adapted from (Donnelly et al., 2014; Turner et al., 2021)

3.6 Surgical implantation

Surgical implantation is an invasive operation in which a body is cut open with medical instruments so that an implant can be placed inside the body. Invasive methods often have more risks and cause more discomfort to the patient compared to non-invasive methods. (J. Li & Mooney, 2016) However, in some cases, surgical implantation is the only method that can offer desired results. Compared to for example intravenous injections, surgical implantation does not require frequent hydrogel administrations. Thus, the dose size decreases, and the treatment is more efficient. (Shen et al., 2018) In addition, efficacy increases because implanted hydrogels often have larger contact areas with the tumor mass (Ding et al., 2011). Surgical implantation of hydrogels is often combined with a patient's tumor resection surgery. In this case, the hydrogel works as a post-surgery application and its function is to reduce the risk of cancer recurrence. (Askari et al., 2020) Various cancer types, such as gastric cancer and breast cancer, have been treated with surgically implanted hydrogels (Askari et al., 2020; H. Zhang et al., 2016).

The design of implantable hydrogels can be exact because the hydrogel can be delivered in its final form. Three-dimensional printing can be utilized in the manufacturing process of implantable hydrogels. The technique is excellent in making mechanically strong hydrogels and it allows the design of complex geometries. (Askari et al., 2020) The size of macroscopic gels is best suited for surgical implantation (J. Li & Mooney, 2016).

4. DIAGNOSTIC APPROACHES USED WITH THERANOSTIC HYDROGELS

In addition to cancer therapy, theranostic platforms offer tools for the diagnosis of cancer. The imaging agents can be entrapped into hydrogel structure either physically or chemically. (F. Wang et al., 2021) The aim of the diagnostics is usually to get information about hydrogel degradation or cancer cells. In the latter, the imaging process usually starts with a chemical or biological recognition and interaction formation between the imaging agent and the target. For instance, the imaging agents can accumulate at the cancer site and attach to the cancer cell surface or penetrate through the cell membrane with the help of enhanced permeability and retention of cancer cells. This so-called “self-assembly event” is followed by a “readout event”, which utilizes a device outside of the body in the detection of the imaging agent complex. (Caballero et al., 2022; Hurst Petrosko, 2011, p. 5-6)

Hydrogel theranostic platforms have many advantages in diagnostics. Compared to other diagnostic strategies, hydrogel theranostic platforms enable repetitive and continuous monitoring of cancer and pharmacokinetics. (F. Wang et al., 2021) Compared to for example intravenous injections of imaging agents, theranostic hydrogels provide long-term diagnostics (Kim et al., 2011). Longevity is achieved by a hydrogel that can trap the agents inside of its structure and prevent degradation (Caballero et al., 2022). The platform may also compensate for the need for surgery and biopsy collection, and thus the price of the treatment is reduced, and the risk associated with potential invasive methods can be avoided (Caballero et al., 2022; Jeyamogan et al., 2021). Furthermore, the utilization of theranostic platforms offers more personalized treatment for the patients. For example, so-called adaptive targeting can be implemented, referring to a therapy technique that exploits real-time information in the alteration of cancer treatment. Adaptive targeting can respond to individual changes that may occur in the tumor or the tumor surroundings. (Hurst Petrosko, 2011, p. 415-416). Besides the ability to detect cancer cells and discover tumor recurrence, theranostic platforms facilitate efficacy evaluation of the therapeutic agents. The evaluation can be achieved by examining the tumor size, drug biodistribution, or drug uptake by the cancer cells. (Shetty et al., 2019) With the help of theranostic hydrogels also the safety of the treatment can be evaluated, and the treatment response can be estimated. Moreover, the imaging agents can be useful in monitoring the degradation and formation of the hydrogel. (F. Wang et

al., 2021) The above-mentioned monitoring is convenient in estimating the therapeutic effect and assuring that the hydrogel has been administered successfully into the body (Dong et al., 2021; Jin et al., 2019).

In this chapter, four common diagnostic approaches are introduced: optical, magnetic, nuclear, and ultrasound imaging. The aim is to briefly introduce the physics that are essential in understanding the mechanism but mainly concentrate on the aspects that are important in understanding hydrogel theranostic platforms. Even though the imaging techniques are presented separately, multiple imaging agents from different categories can be incorporated into the same platform. In fact, these hybrid platforms often offer better resolution and sensitivity than platforms that only utilize one diagnostic type. (Shetty et al., 2019)

4.1 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is utilized to visualize the anatomical structures of soft tissue (Z. Gao et al., 2016). MRI is a common imaging technique due to its high spatial resolution and non-ionizing properties (Lock et al., 2017; F. Wang et al., 2021). However, compared to some other imaging techniques, MRI is considered to have low sensitivity, long scan times, and relatively high cost (Dong et al., 2021). The MRI technique examines the alignment of protons in the body. Water molecules have two protons in their structure, thus the MRI examines the alignment of water molecules. Due to different water contents, different tissue types have distinctive features in MRI images. Before a magnetic field is introduced, protons in the body are spinning on their randomly aligned axes. The magnetic field affects the alignment of the protons and therefore the protons are lined-up under the influence of the magnetic field. This alignment also induces the generation of a magnetic vector. After applying the magnetic field, a radio wave frequency is introduced, and protons are pulled against the magnetic field. MRI scanner can detect signals that are emitted when radiofrequency is switched off and protons are returned to a relaxed state. In the relaxed state, the protons are aligned again with the magnetic field. The time of relaxation has major importance in the visualization of MRI results. T_1 defines the time that takes for a magnetic vector to recover to its resting state, whereas T_2 refers to the duration for the axial spin to return to its resting state. In other words, T_1 is the time taken for longitudinal relaxation and T_2 is the time taken for transverse relaxation. The relaxation time is dependent on the nature of the molecules and the environment. Different tissue types have different relaxation times and in the MRI images, the differences can be seen in the varying intensity. (Berger, 2002)

Hydrogel theranostic platforms have to utilize MRI contrast agents in the imaging process because otherwise the background water molecules would interfere with the ones in the hydrogel. Nanoparticles are advantageous in cancer diagnostics because they emphasize and detect tumor tissue even better. (Z. Gao et al., 2016; F. Wang et al., 2021) MRI contrast agents affect the relaxation of protons. They can affect either T_1 or T_2 relaxation time and thus affect the signal intensity seen on the MRI pictures. T_1 contrast agents can be also called positive contrast agents and they accelerate the longitudinal relaxation. T_2 contrast agents or so-called negative contrast agents speed up the process of transverse relaxation. Contrast agents can be categorized based on their actions. Paramagnetic contrast agents adapt the value of T_1, and superparamagnetic contrast agents have an influence on T_2. Gadolinium-chelates are a typical example of paramagnetic contrast agents whereas ferrite nanoparticles are a typical example of superparamagnetic contrast agents. (Z. Gao et al., 2016; Kim et al., 2011) Recently, the toxicity of gadolinium-chelates has provoked conversation (F. Wang et al., 2021). The physical, chemical and biological properties of nanoparticles can be manipulated in order to achieve desired results. The surfaces of magnetic nanoparticles are often modified and tumor-specific bioligands are conjugated to them to ensure better attachment at the tumor site. (Z. Gao et al., 2016)

For instance, Kim *et al*. (2011) developed a poly(organophosphazene) hydrogel that was embedded with cobalt ferrite nanoparticles and paclitaxel. Paclitaxel worked as the anticancer drug whereas $CoFe_2O_4$ worked as the T_2 imaging agent. The function of $CoFe_2O_4$ was to penetrate the tumor mass for contrast enhancement. The surface of $CoFe_2O_4$ was modified with PEG to ensure low cytotoxicity. During the three weeks, a notable decrease in tumor volume could be observed in magnetic resonance images.

4.2 Optical imaging

Optical imaging exploits light to investigate the properties of tissues, organs, and molecules. Optical imaging utilizes non-ionizing radiation such as visible, ultraviolet, and infrared light. (Pansieri et al., 2019) Optical imaging has high sensitivity, but the technique suffers from poor tissue penetration (Dong et al., 2021). Optical imaging can be divided into many different categories, for example Raman spectroscopy, fluorescence imaging, and optical coherence tomography are typical optical imaging techniques (Chen et al., 2018; Rosenthal & Zinn, 2010).

Compared to other optical imaging subtypes, fluorescence imaging is the most used diagnostic technique in this application. It has a low cost, high spatial resolution, and it enables real-time monitoring. (Kosaka et al., 2009; F. Wang et al., 2021) In the first step of fluorescence imaging, molecules are targeted with light, usually near-infrared (NIR) light due to its better photon penetration compared to visible light (Dong et al., 2021). Fluorescence molecules, or “fluorophores”,

absorb the light and in response the molecules emit light that has a longer wavelength compared to the absorbed light. The phenomenon that is exhibited by fluorophores can be explained by their electron movements. Before the molecule has absorbed the energy of the photon, the electrons are in a ground state. After the absorption, the electrons move to an excited state. Then, some of the energy is released into the environment and part of it is released in the form of an emitted photon. Due to the energy released to the environment, the emitted light has less energy and a longer wavelength than the absorbed photon. (Rosenthal & Zinn, 2010, p. 4-5). Fluorescent agents can accumulate at the tumor site with the help of a targeting ligand (Kosaka et al., 2009). The composition of the targeting ligand varies a lot, but their goal is the same. Targeting ligands aim to create an association between the fluorophore and the target molecule. The complex that consists of the targeting ligand and the fluorophore can be referred to as the fluorescent probe. (Rosenthal & Zinn, 2010, p. 60) Moreover, fluorescence imaging renders possible the tracking of multiple fluorescent signals at the same time. The ability is enabled by fluorescence molecules that can emit light with distinctive wavelengths. This feature is especially advantageous when multiple auto-fluorescent drugs are administered to the body. (Sheng et al., 2021)

Fluoresceine and carbon nanodots are common fluorophores. Fluorescein can emit and absorb light in the visible light range (400-700 nm), whereas carbon nanodots emit and absorb red light (620-750 nm). (Kohli et al., 2019; F. Wang et al., 2021) In addition to the above-mentioned fluorescence tags, also some antitumor drugs have fluorescence abilities (Sheng et al., 2021). In fact, also some hydrogels can display autofluorescence without incorporating a fluorescence tag. In particular, hydrogels that have carbonyl groups in their structure have the ability for autofluorescence. However, this property provides information mostly on the gelation process of hydrogels and thus does not have a big significance in cancer diagnostics. (H.-X. Xu et al., 2019)

Sheng *et al*, (2021) generated a hydrogel consisting of PEG and polycaprolactone. The hydrogel was loaded with doxorubicin and curcumin, which showed a therapeutic effect on the tumor, while being fluorescent. The fluorescence imaging enabled a precise administration of the hydrogel platform, tracking of drug release, and evaluation of chemotherapeutic effects. The tracking was carried out with laser confocal microscopy. In addition to the hydrogel platform, the therapeutic agents were also administered to one mice group without incorporating them into a drug delivery system. According to the results, the group that was treated with the hydrogel system inhibited tumor growth better.

4.3 Nuclear imaging

Like optical imaging, also nuclear imaging is based on electromagnetic radiation. However, separating from optical imaging, nuclear imaging utilizes ionizing radiation which has a shorter

wavelength. The photons of ionizing radiation have more energy and thus they may cause harmful effects on the living body. Nuclear imaging can be categorized into three groups: X-ray computed tomography (CT), positron emission tomography (PET), and single-photon emission computed tomography (SPECT). Nevertheless, these techniques are usually combined to achieve more precise results. PET and SPECT are rarely used on their own and they are usually combined with CT to increase the specificity. (F. Wang et al., 2021)

Radioactive tracers are essential in SPECT and PET as they emit radiation that can be detected with a scan. These radioactive tracers are usually bound to carrier molecules that transport the complex to a target site. Thereafter the radioactive tracers start to decay and emit γ-ray. The emission of γ-ray happens differently depending on whether the imaging technique is SPECT or PET. The radioactive tracers used in PET produce positrons when they start to decay. Eventually, the positron collides with an electron. Consequently, energy is released in the form of two γ-rays that travel in opposite directions. The most popular radioactive tracer used in PET is fluorine-18. It has a half-life of only 1,83 hours. Different from PET, the radioactive tracer of SPECT produces a single photon. The most popular radioactive tracer of SPECT is technetium-99m. It has a half-life of six hours and thus it is rapidly cleared from the body. Additionally, it emits γ-ray energy of 140 keV which provides efficient imaging while still being safe for patients. (Hamoudeh et al., 2008)

In CT, X-rays that are generated in an X-ray tube are directed towards a target from multiple orientations. CT scanners are located opposite the radiation source, and they can detect radiation that has not been absorbed by the tissues. The measurements are processed on a computer to produce cross-sectional images. CT can also utilize contrast agents to highlight the target areas. Substances that have high density or high atomic numbers are excellent CT contrast agents because they can efficiently absorb X-rays. For instance, iodine and barium have these characteristics and are therefore excellent contrast agents for CT. (Lusic & Grinstaff, 2013)

Y. Wu *et al.* (2018) developed a thermoresponsive poly(*N*-acryloyl glycinamide-*co*-acrylamide) hydrogel that contained doxorubicin and polydopamine coated-gold nanoparticles. The polydopamine coated-gold nanoparticles had a significant role in not only photothermal therapy but also in the diagnostics of breast cancer. The NIR light-induced photothermal effect gave rise to gel-sol transition and release of doxorubicin. Gold nanoparticles are also excellent CT contrast agents due to their high atomic number. The gold nanoparticles brightened the location of the hydrogel in CT images and thus enabled a clear recognition of the hydrogel position.

Another study conducted by Y. Wang *et al.* (2020) investigated a dopamine conjugated poly(α,β-aspartic acid) and $FeCl_3$ hydrogel system in cancer theranostics. $Na^{188}ReO_4$- loaded hydrogel could exhibit synergistic treatment as the platform could offer photothermal and radioiso-

tope therapy. Radioisotope therapy is based on radionuclides that can damage target cells with minimal damage to surrounding tissue. In this study, the hydrogel contained ^{188}Re radionuclide which can emit β-and γ-rays. ^{188}Re has also significance in diagnostics. With SPECT and CT, it was possible to investigate the locations of the radionuclides. According to the results, the ^{188}Re containing hydrogel enabled a better accumulation of the ^{88}Re at the tumor site compared to free ^{188}Re.

4.4 Ultrasound imaging

Ultrasound is a versatile tool when it comes to hydrogel theranostic platforms. On top of its excellent capability to trigger drug release, ultrasound can be utilized in the diagnosis of cancer. It is good to note that even though ultrasound is a widely used diagnostic tool in general and in cancer detection, it is not yet as commonly employed in the diagnostics of hydrogel theranostic platforms. However, an imaging technique that utilizes both optical and ultrasound imaging has been widely under research.

Photoacoustic imaging (PA) is an imaging modality that utilizes both ultrasonic waves and electromagnetic radiation. Hence, it combines the best parts of optical and ultrasound imaging. For instance, photoacoustic imaging has a high spatial resolution which is a characteristic feature of optical imaging, and good penetrability which is usual for ultrasound. (Jin et al., 2019; F. Wang et al., 2021) In photoacoustic imaging, acoustic waves are not directed towards the target from the outside but are generated inside of the body. The generation of acoustic waves is triggered by light, usually NIR light, and can be explained by a phenomenon called the photothermal effect. The photothermal effect is based on photothermal transduction agents that can convert energy from light to heat. Photothermal therapy is a cancer treatment technique that utilizes the photothermal effect in destroying cancer cells. The increasing temperature causes the death of cancer cells. The photothermal effect has also the ability to generate acoustic waves which makes it so advantageous in diagnostics. The emitted acoustic waves can be detected outside of the body and converted into imaging signals. Thus, the photothermal transduction agents can provide cancer therapy and diagnostics simultaneously. The ideal photothermal transduction agents accumulate in tumors, are biocompatible and biodegradable, have high photothermal conversion efficiency, and have different absorption wavelengths than surrounding tissue. The photothermal transduction agents can be inorganic or organic materials. Organic NIR-responsive small molecules and semiconducting polymers are very biocompatible and biodegradable, whereas inorganic materials such as noble metals often have higher photothermal conversion efficiency. (Y. Liu et al., 2019)

Jin *et al.* (2019) developed a polypeptide-based hydrogel that was loaded with chemotherapeutic paclitaxel and photothermal Ag_2S quantum dots. Photothermal- and chemotherapy successfully suppressed the growth of ovarian carcinoma tumors. Photoacoustic and fluorescence imaging provided real-time information on the degradation of hydrogels which also facilitated a better understanding of the therapeutic effect of the theranostic platform.

5. EXAMPLE OF HYDROGEL THERANOSTIC PLATFORM

In this chapter, a hypothetical example of a hydrogel theranostic platform is presented. Even though the aim is to design the most competent platform, due to unique advantages and disadvantages it is impossible to unambiguously choose a superior platform. It must be also considered that in theory the chosen platform possesses some outstanding features, but it is challenging to extrapolate how the platform would perform in practice. The presented hypothetical platform is pH- and thermosensitive injectable poly(N-isopropyl acrylamide-co-acrylic acid) hydrogel, loaded with doxorubicin and gold nanoparticles. Multiple aspects of the platform will be considered: the release mechanism, the delivery method, the therapeutic approach, and the diagnostic technique.

Due to the photothermal effect of gold nanoparticles, the temperature within gold particle laden hydrogel will increase under light exposure. Therefore, hydrogels based on thermosensitive polymers can effectively control the release of the embedded agents. PNIPAM is a widely investigated thermosensitive polymer that has an LCST of about 32°C. PNIPAM can be copolymerized with acrylic acid to improve its mechanical properties. There is a large number of carboxylic groups in the structure of acrylic acid and therefore the polymer is pH sensitive. The usage of pH-sensitive hydrogels in cancer treatment is logical because the tumor microenvironment is acidic. The pH sensitivity of the hydrogel is beneficial because it enhances the release of drugs and imaging agents. The copolymerization of acrylic acid with PNIPAM also elevates the LCST of the hydrogel. Despite the elevation of LCST, the embedded substances are released efficiently due to high temperatures caused by the photothermal effect. (X. Gao, Cao, et al., 2013; Rana & de La Hoz Siegler, 2021)

Injection could be employed for this hydrogel as it is a minimally invasive and widely researched delivery method. The injection also enables the treatment of various cancer types. Due to thermosensitive features of poly(N-isopropyl acrylamide-co-acrylic acid) hydrogel, it can utilize *in situ* gelling when injected. Before the hydrogel is injected into a body, the hydrogel is in a solution state because the temperature does not reach its LCST. Whereas after injection, the temperature rises above its LCST, and hydrogel transforms into a gel state.

The hydrogel platform contains doxorubicin and gold nanoparticles and thus the platform exhibits synergistic chemo- and photothermal effects. Doxorubicin is a widely used antitumor drug whereas gold nanoparticles are common photothermal transduction agents. The combination

therapy has shown significantly better results than utilizing the treatments separately. For example, it may help overcome the problems associated with multi-drug resistance of tumor cells. Multi-drug resistance is a cellular defense mechanism, which causes the ineffectiveness of chemotherapy drugs. (Huang et al., 2022)

In addition to the therapeutic characteristics, gold nanoparticles have also significance in diagnostics. Gold nanoparticles can act as contrast agents in PA imaging and CT. They can emit acoustic waves under light exposure which makes them excellent contrast agents for PA imaging. Compared to many other photothermal transduction agents, they also have excellent photothermal conversion efficiency. For comparison purposes, the nanoparticles can be targeted with different wavelengths of light during the research process. The size and the shape of the gold nanoparticles determine which wavelength can be absorbed. For instance, the particles can be in the shape of rods, shells, or rings. Despite the size and shape of the gold nanoparticles, the best results can be achieved with NIR or red light because of their great penetration capability. In addition to the ability to absorb light, gold nanoparticles can absorb x-ray radiation, which makes them suitable contrast agents for CT. To improve biocompatibility, gold nanoparticles are coated with glycol-chitosan. Glycol-chitosan-coated gold nanoparticles have shown promising results in previous studies, as they have also successfully enhanced tumor accumulation. The predicted and desired outcome is that glycol-chitosan coated gold nanoparticles are partially taken up by the cancer cells and the cancer cells could be visualized in the diagnostic images. In a successful treatment, subsequent imaging would reveal the destruction of cancer cells. (Y. Liu et al., 2019; I.-C. Sun et al., 2019)

In conclusion, it is preferred that multiple factors are responsible for the essential functions of the hydrogel such as the release, therapy, and diagnosis. For instance, the release of the doxorubicin and gold nanoparticles is mediated by both pH and temperature. In addition, the released agents offer synergistic chemo-photothermal therapy. Furthermore, CT and PA imaging provide combined diagnostics. When multiple factors mediate the release, therapy, and diagnosis, the platform is more adaptable to changes. However, a too complicated platform is hard to manufacture, and its effects may be difficult to predict. The main reason why this hydrogel platform remains simple but still versatile is that it incorporates multipurpose gold nanoparticles into its structure.

6. CONCLUSION

Due to the severity and high prevalence of cancer, new strategies for cancer treatment and diagnostics are required. Theranostic hydrogels offer innovative and personalized methods to fight cancer while also taking into account patient comfort. They combine therapy and diagnosis on a single platform which enables real-time monitoring of the tumor site. This thesis has gathered information on some of the most relevant stimuli-responsive hydrogels, discussed the methods for delivering hydrogels into the body, and summarized the diagnostic techniques that can be exploited to obtain data from the cancer site. The utilization of stimuli-sensitive hydrogels renders possible the controlled and simultaneous release of drugs and imaging agents. Especially hydrogels that are sensitive to multiple stimuli have shown promising results as they are more adaptable and efficient compared to hydrogels sensitive to a single stimulus. However, there are few issues with different stimuli-sensitive hydrogels. For instance, thermosensitive hydrogels often lack mechanical strength, and due to insufficient penetration ability of light, the function of photosensitive hydrogels may be affected. Nonetheless, these difficulties can be overcome by further research and using each stimuli-responsive hydrogel in its suitable environment. In the case of different delivery methods, new research is especially needed when it comes to other methods than injection. With the intention of choosing the most adequate delivery method, the cancer location, invasiveness of the technique, hydrogel size, and other hydrogel properties such as bioadhesiveness must be taken into consideration. When comparing different diagnostic approaches for theranostic hydrogels, the most prominent differences can be seen in the cost and effectiveness of the method. Moreover, some drawbacks, such as toxicity of some MRI agents and radiation exposure of nuclear imaging must be considered when selecting the most suitable imaging tool. The obstacles related to theranostic hydrogels are partially solved but still need to be overcome completely before moving them from preclinical to clinical trials. Overall, there is an abundance of potential research targets in the field of theranostic hydrogels that have a promising outlook in cancer treatment and diagnostics.

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