NTNU – Trondheim
Norwegian University of
Science and Technology

The Effect of G-block on the Extracellular Matrix Barrier to Molecular Diffusion

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Biotechnology (5 year)
Submission date: May 2015
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Preface

This master thesis was conducted at the Institute of Biotechnology at the Norwegian University of Science and Technology (NTNU) from September 2014 to May 2015. The thesis was written in collaboration with a research group led by Professor Kurt Ingar Draget.

Throughout this year I have experienced the challenges of research, but also how fulfilling it is to overcome them. I have obtained new knowledge regarding practical laboratory work, critical thinking and scientific writing. I would like to thank my supervisors, Professor Kurt I. Draget and Dr. Catherine Taylor Nordgård for the opportunity to work with a topic I find very interesting and important for the future. They have provided excellent guidance and support, both regarding theoretical and practical issues. I would also like to thank Morten Johnsen Dille for technical support, especially in the start-up process of the practical work. Furthermore, I would like to thank my fellow master students for moral support and comfort through this process. It has been valuable to discuss with someone in the same situation. Lastly, I would like to thank my supporting friends and family for encouragement, motivation and for always believing in me.

Trondheim, May 2015

Stine Malene Hansen Wøien

Abstract

Changes in extracellular matrix (ECM) are one of the characteristics of solid tumours. Many types of cancers associated with high mortality rates develop solid tumours with an extensive fibrotic extracellular matrix. Over-expression of ECM may result in an increased barrier to drug diffusion in the tumour interstitium, resulting in inefficient drug delivery. There is an increasing number of studies supporting ECM as a potential target of treatment to improve drug delivery in tumours. RiXOVA is an anti-cancer drug candidate based on G-block technology, targeting extracellular matrix. Experimental *in vivo* data has shown reduction in tumour growth in a mouse model of pancreatic cancer with RiXOVA both as a single agent and in combination with the standard cytostatic gemcitabine. A hypothesis for the mode of action of RiXOVA is that G-block is able to reduce the extracellular matrix barrier to drug diffusion in tumours with an over-expression of ECM, such as pancreatic cancer, resulting in improved drug and immune system access to the cancer cells.

The aim of this master thesis was to investigate if G-block can reduce the extracellular matrix barrier to molecular diffusion. A method for studying diffusion of model compounds out of an extracellular matrix was developed and optimized with Matrigel as model for ECM and the dye tartrazine and Alexa Fluor® 488 IgG as model compounds. By utilizing the developed method, diffusion of the model compounds from Matrigel with and without the presence of G-block was investigated. In addition, the rheological profile of Matrigel with and without G-block was characterized to study the effect of G-block on the rheological properties of Matrigel.

G-block demonstrated an increased diffusion rate of both tartrazine and IgG from Matrigel. From the rheological measurements, G-block did not demonstrate an effect on the rheological profile of Matrigel in the presence of saline and tartrazine. However, rheological data demonstrated a reduction in the elastic properties of Matrigel with G-block as the only added component. It was postulated that G-block reduces the extracellular matrix barrier to molecular diffusion by affecting a bound fraction of tartrazine and IgG in Matrigel. It was also suggested that G-block can reduce the steric hindrance by affecting the structure of the Matrigel network, including the elastic properties. However, due to the complex nature of Matrigel, an explanation of the interactions between Matrigel and G-block is most likely multifactorial.

Sammendrag

Endringer i ekstracellulær matriks (ECM) er en av flere kjennetegn ved solide tumorer. Flere krefttyper assosiert med høy dødsrate utvikler solide tumorer med en omfattende fibrotisk ekstracellulær matriks. Overekspresjon av ECM kan resultere i en økt barriere for medisindiffusjon i tumor interstitium, som igjen kan føre til ineffektiv medisinlevering. Antallet studier som støtter ECM som et potensielt behandlingsmål for å forbedre medisinlevering i tumorer øker stadig. RiXOVA er en antikreft legemiddelkandidat basert på G-blokk teknologi rettet mot ECM. Eksperimentell *in vivo* data har vist reduksjon i tumorvekst i mus med bukspyttkjertelkreft både med RiXOVA administrert alene og i kombinasjon med et standard cytostatikum (gemcitabine). En hypotese for mekanismen bak RiXOVA er at G-blokk kan redusere ekstracellulær matriks barrieren for medisindiffusjon i tumorer med overekspresjon av ECM, som kan føre til økt tilgjengelighet av medisin og immunsystemkomponenter i tumorer.

Målet med denne masteroppgaven var å undersøke om G-blokk kan redusere ekstracellulær matriks barrieren for diffusjon av molekyler. En metode for å studere diffusjon av modellforbindelser ut av en ekstracellulær matriks ble utviklet og optimalisert med Matrigel som modell for ECM og fargestoffet tartrazine og Alexa Fluor® 488 IgG som modellforbindelser. Den utviklede metoden ble benyttet til å studere diffusjon av modellforbindelser fra Matrigel med og uten G-blokk. Den reologiske profilen til Matrigel med og uten G-blokk ble karakterisert for å studere effekten av G-blokk på de reologiske egenskapene til Matrigel.

G-blokk demonstrerte en økt diffusjonshastighet av både tartrazine og IgG fra Matrigel. De reologiske målingene viste at G-blokk ikke hadde en effekt på den reologiske profilen til Matrigel med saline og tartrazine tilsatt. Reologiske data viste en reduksjon i de elastiske egenskapene til Matrigel med kun G-blokk tilsatt. Det ble postulert at G-blokk kan redusere ECM barrieren for molekyldiffusjon ved å påvirke en bundet fraksjon av tartrazine og IgG i Matrigel. En hypotese om at G-blokk kan redusere den steriske hindringen i gelen ved å påvirke strukturen til Matrigel nettverket ble også foreslått. Det ble imidlertid konkludert med at en forklaring på interaksjonene mellom Matrigel og G-blokk involverer flere faktorer på grunn av Matrigels komplekse karakter.

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1. Introduction

1.1 Scientific introduction

1.1.1 Extracellular matrix

The extracellular matrix (ECM) fills the volume between cells in tissues and is a complex network of different proteins and polysaccharides. There are two main classes of macromolecules constituting the extracellular matrices in animal tissues; fibrous proteins and a group of polysaccharides called glycosaminoglycans (GAGs). ECM is composed of different protein fibers embedded in a hydrated gel consisting of a network of GAG (Alberts *et al.*, 2008).

GAGs are unbranched anionic polysaccharides with a repeating disaccharide unit made up of an amino sugar and a uronic acid. All classes of GAGs, except for hyaluronan, are usually covalently linked to core proteins to form proteoglycans. Proteoglycans attract counterions due to negative charges in the molecules, resulting in an increased osmotic pressure and diffusion of water into the matrix. The swelling of the matrix as it absorbs water explains the large volume ECM fills in tissues. Fibrous or fiber-forming proteins comprise a wide range of proteins that provide the strength and elasticity of ECM. Tensile strength is provided by fibrillar collagens of five different types. The collagens are triple-stranded helical molecules assembled into long fibrils. Elastin molecules provide elasticity by forming a flexible network of fibers and sheets. Fibrous proteins also serve as anchors to cells in ECM, often in combination with large multidomain glycoproteins (Alberts *et al.*, 2008).

Different forms of ECM generally contain the same macromolecules, but vary in organization and quantity of the different classes of molecules. The composition of ECM may differ between tissues and animal phyla, but also within the same tissue. The various types of structure give rise to several types of ECM with different properties. The animal ECM includes both the basal lamina or the basement membrane and the interstitial connective tissue matrix, which differ in biochemistry and morphology. The basal lamina is a specialized form

of extracellular matrix found in all multicellular animals. It consists of extracellular macromolecules assembled in a thin, though and flexible sheet. The glycoprotein laminin is the main component and forms the basis for the two-dimensional sheet structure. Specific interactions between laminin, type IV collagen, the glycoprotein nidogen and the proteoglycan perlecan result in the structure of the mature basal lamina. This sheet can be organized in several ways. The basal lamina sheet underpins all epithelia, separating the epithelial tissue from the connective tissue. Basal lamina also surrounds specific cell types, such as fat and muscle cells, and can be placed between two cell sheets, thereby serving as a filter. The functions of the basal lamina are many and varied. In addition to mechanical support, filtering and a barrier between epithelia and connective tissue, this flexible sheet is able to determine cell polarity, participate in the organization of proteins in neighboring plasma membranes and influence cell metabolism and migration. Promotion of cell proliferation, differentiation and survival are also among the functions of the basal lamina (Alberts *et al.*, 2008).

Connective tissues contain more varied and bulky forms of ECM than the basal lamina. The interstitial connective tissue matrix is composed of the same type of macromolecules as the basal lamina; glycosaminoglycans in the form of proteoglycans and fibrous proteins. However, the organization and quantity vary from that of the basal lamina. The macromolecule components of ECM in connective tissues are secreted by fibroblast cells, which are localized in the matrix. The interstitial connective tissue matrix binds cells in connective tissues together and provides physical support, in addition to affect the cell shape, development, survival, polarity and migration (Alberts *et al.*, 2008).

1.1.2 Extracellular matrix and the tumour microenvironment

One of the challenges with cancer treatment is the delivery of therapeutic drug molecules to target tumour cells. Firstly, the drug has to be effective against the tumour cells *in vivo*. Secondly, the drug molecules must reach the target cells in vivo in sufficient amounts in order to exert a therapeutic effect. A drug can therefore be highly effective *in vitro*, but might never reach its target *in vivo*. Transport of drug molecules from the blood to the target cells in the tumour interstitium involves several steps and various biological barriers. Drug molecules administered and distributed in the circulatory system must enter the tumour vasculature, cross the microvascular wall and migrate through the tumour interstitium and microenvironment to reach the target cells. Diffusion and convection are processes involved in this transport. The transport of therapeutic agents is often more challenging in tumour tissue, as tumours have been shown to develop in ways that hinder one or several of the transport steps mentioned above. Impeding of drug delivery may result in partial or total tumour drug resistance (Jain, 2012).

One of the biological barriers possibly contributing to drug resistance of solid tumours is the extracellular matrix. Drug molecules have to penetrate the tumour microenvironment in order to reach the target cells. Over-expression of ECM may lead to resistance to drug diffusion through the tumour interstitium, resulting in inefficient drug delivery (Sriraman *et al.*, 2014). Some types of cancer, such as the pancreatic cancer ductal adenocarcinoma, develop solid tumours with an extensive fibrotic desmoplasmic stroma consisting of cancer-associated fibroblasts, blood vessels, immune cells and extracellular matrix components. The organization of desmoplasmic stroma surrounding pancreatic cancer cells is presented in Figure 1-1. The main type of pancreatic cancer, ductal adenocarcinoma, is associated with a high mortality rate primarily due to development of mechanically hard tumours, which are able to resist most of today's cancer therapies (Olson and Hanahan, 2009).

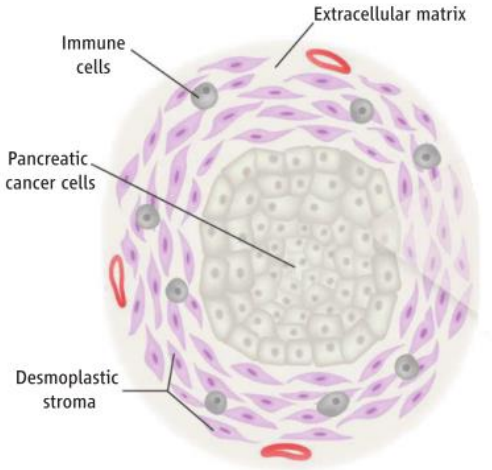


Figure 1-1. Pancreatic cancer cells enveloped by desmoplasmic stroma composed of fibroblasts, immune cells, fibroblasts and extracellular matrix macromolecules. Obtained from (Olson and Hanahan, 2009).

Studies have shown the importance of tumour microenvironment in tumour progression and drug delivery. Cancer-associated fibroblasts in desmoplasmic stroma have proven to play an important role for the poor vascularization in pancreatic tumours. Few blood vessels in addition to cancer cells enveloped in an extensive fibrotic stroma contribute to an increased barrier to drug diffusion. Removal of fibroblast has demonstrated an increase in the generation of new blood vessels, in addition to improved drug delivery and longer life span in a *de novo* mouse model (Olive *et al.*, 2009). There is an increasing number of studies supporting ECM as a potential target of treatment to improve drug delivery in cancer treatment. In a study of passive diffusion of macromolecules through the tumour interstitium, Netti *et al.* found a correlation between transport resistance and mechanical stiffness of ECM, suggesting ECM as a transport barrier preventing penetration of drug molecules in solid tumours. Diffusion of the macromolecule immunoglobulin G (IgG) was studied in four different tumour lines: human colon adenocarcinoma (LS174T), human glioblastoma (U87), human soft tissue sarcoma (HSTS 26T) and murine mammary carcinoma (MCaIV). The results showed that the diffusion of IgG was greater in MCaIV and LS174T than in U87 and HSTS 26T. Furthermore, the U87 and HSTS 26T tumours were found to have a higher content of collagen than the other tumours, in addition to well-ordered collagen-proteoglycan-linked matrices (Netti *et al.*, 2000). The results of the study show a correlation between resistance to IgG diffusion, tumour rigidity and arrangement of collagen in ECM.

1.1.3 Matrigel

In this thesis, Matrigel mimicked tumour ECM in the study of molecular diffusion through ECM. Matrigel is a soluble extract from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma that gels at room temperature to form a reconstituted basement membrane. The main components of Matrigel are the ECM proteins abundant in EHS tumours; laminin, collagen IV, nidogen and the proteoglycan heparan sulfate, in addition to several growth factors that are naturally present in EHS sarcomas. Studies have shown that the main protein components of the EHS tumour matrix interact to form a hydrogel with a lamellar structure resembling the structure of basement membranes (Kleinman *et al.*, 1986, Kleinman *et al.*, 1982, Hughes *et al.*, 2010). Matrigel is widely used in cell culture and cancer research. Its main areas of application are imitation of extracellular matrix in cancer and stem cell culture and growth of cells that are sensitive to culture conditions. Matrigel is effective for attachment and differentiation of different cell types, including normal and transformed anchorage dependent epithelioid, human embryonic stem cells, neurons, vascular endothelial cells and hepatocytes (Hughes *et al.*, 2010, Corning(A))

1.1.4 Diffusion

A concentration gradient is the driving force of diffusion; the movement of molecules from a region of high concentration to a region of lower concentration (Edward, 1970). Gel diffusion studies are used to investigate solute transport in gels and can also provide information about the gel network structure. A diffusion coefficient is often determined to predict the solute diffusion (Westrin *et al.*, 1994). The diffusion coefficient, D, can be found using the Stokes-Einstein equation:

$$D = kT/6\pi\eta r \qquad \textbf{(1-1)}$$

where k is the Boltzman coefficient, T is the temperature in Kelvin, η is the viscosity of the medium and r is the radius of the solute particle (Edward, 1970, Muhr and Blanshard, 1982).

1.1.5 Alginates

Structure

Alginates are a family of linear copolymers occurring as main structural components of marine brown alga (seaweeds) and as capsular polysaccharides in soil bacteria. They consist of chains with (1→4) linked β-D-mannuronate (M) and α-L-guluronate (G) in a complex and varying sequence. Alginate is usually described as a block polymer as three different block types arise from the distribution of M and G in alginate chains: G-blocks, M-blocks and MG-blocks. G-blocks and M-blocks refer to homopolymeric stretches of guluronate and mannuronate, respectively. MG-blocks are sequences in which M and G alternate in a strict manner. The structural characteristics of alginate are presented in Figure 1-2. The three blocks vary both in length and relative proportions within an alginate chain, resulting in a different chemical composition and variation in physical properties among different alginates (Smidsrød and Moe, 2008, Smidsrød and Draget, 1996).

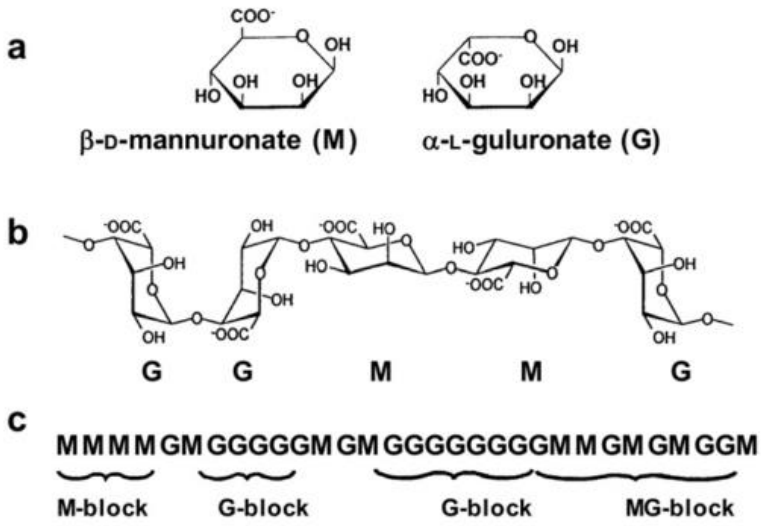


Figure 1-2. Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, (c) block distribution. Obtained from (Draget and Taylor, 2011).

The sequence and amount of M and G in alginate are dependent on the type of organism and tissue from which it is produced. Different bacteria, alga and parts of the alga can produce alginates of different chemical composition (Smidsrød and Draget, 1996). The biosynthesis

starts with the formation of M-residues, followed by conversion of mannuronate monomers to guluronate residues by mannuronan C5-epimerases to form G-blocks or MG-blocks (Smidsrød and Moe, 2008).

Application of alginate

The alginate molecule is a polyelectrolyte known to form gels in the presence of most divalent cations, most commonly Ca^{2+}. The gel forming properties of alginate are related to the content of G-blocks. Gel formation occurs when two or more G-blocks align to create cavities in which divalent cations are selectively bound. Binding of cations in G-block cavities leads to the formation of junctions in a gel network (Figure 1-3). The cavities are formed because of the diaxial glycosidic linkage between guluronate residues caused by the $^1C_4$-conformation of guluronate. Furthermore, cations are bound due to the polyanionic nature of alginate in solution with pH above the pKa value (≈ 4) of the carboxylic acid residues of the uronic acids (Smidsrød and Moe, 2008).

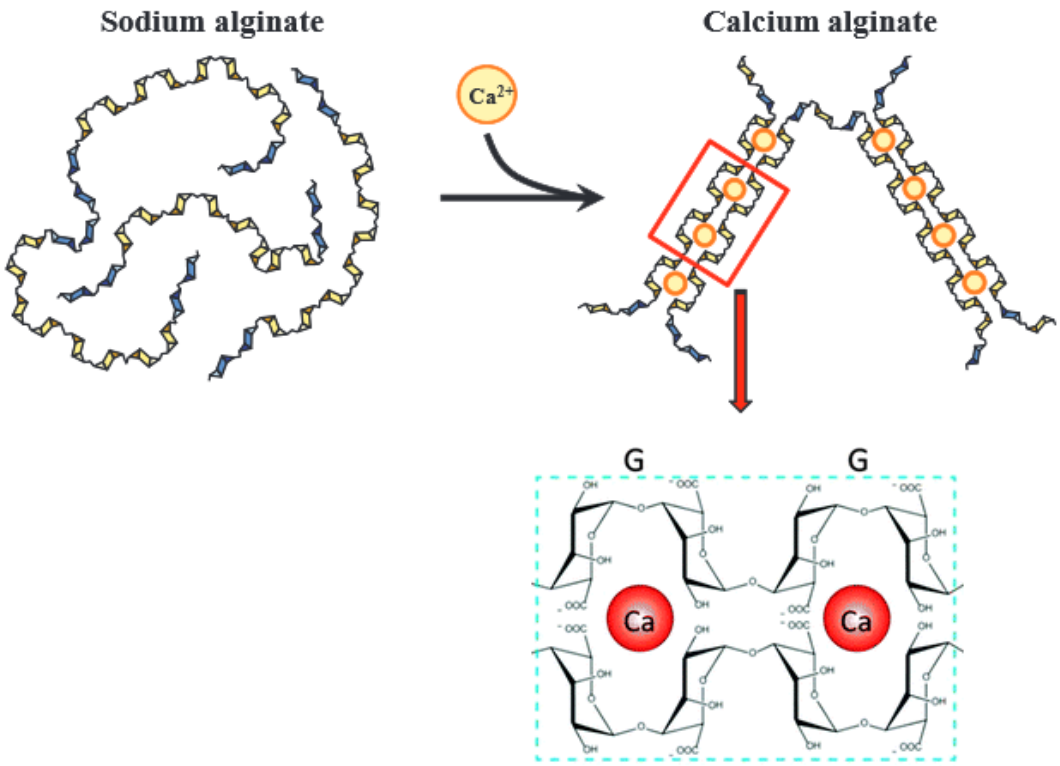


Figure 1-3. Gelation of alginate with calcium ions. A gel network arises as junction zones are formed when the guluronate (G) residues selectively bind the calcium ions. Modified from (Yoon *et al.*, 2014, Kashima and Imai, 2012).

Alginates are widely used industrially because of their ability to retain water and their stabilizing, viscosifying and gelling properties. They are used as stabilizers, film-formers, gelling agents and thickeners in the food, textile and pharmaceutical industry. The fact that alginates are biocompatible and biodegradable makes them beneficial for use in biomedical applications. Traditional areas of application include wound healing, dental impression materials, gastro-enterology and in pharmaceutical formulations as controlled-release mediators and excipients (Onsøyen, 1996, Draget and Taylor, 2011, Draget *et al.*, 1997).

1.1.5.1 G-block

Guluronate oligomers, referred to as G-blocks, are known to alter the gelling kinetics and equilibrium properties of alginate gels (Draget and Smidsrød, 2006). G-blocks have been found to be non-immunogenic, making them suitable for use in biomedical applications (Otterlei *et al.*, 1991).

The ability of G-blocks to alter the mucus barrier function and matrix structure has been demonstrated (Taylor Nordgård *et al.*, 2014). The mucin matrix is classified as a physical gel and is characterized by a dynamic network of polymeric mucin molecules interacting through non-covalent interactions (Taylor *et al.*, 2003). While high molecular weight alginates increase the elasticity and viscosity of mucus, low molecular weight guluronate oligomers (DP 10-DP 20) have been found to reduce the elasticity and viscosity of mucus matrices (Draget, 2011, Nordgard and Draget, 2011). Low molecular weight alginate G-blocks have also been found to improve nanoparticle mobility in mucus matrices and increase the cellular uptake of nanoparticles through a mucus layer. A decrease in the density of network cross-links and increased matrix pore size have been observed after addition of guluronate oligomers to mucus matrices (Figure 1-4) (Taylor Nordgård *et al.*, 2014).

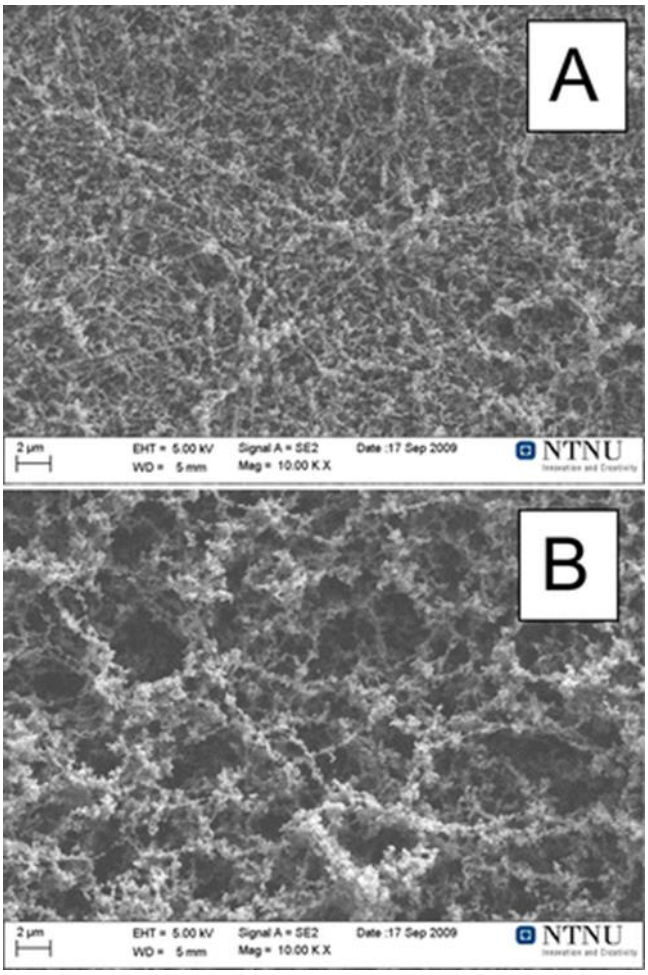


Figure 1-4. Scanning electron microscopy of 20 mg/ml purified pig gastric mucus with (B) and without (A) 4.8 mg/ml guluronate oligomers added. Obtained from (Taylor Nordgård *et al.*, 2014).

Suggestions for the mode of action of G-block on mucus matrices have been postulated. One explanation considers inhibition of interactions between matrix mucins and mobile components. By interfering with mucin-mucin interactions, G-block may also inhibit matrix cross-links, resulting in a reduction in the steric barrier of mucus (Taylor Nordgård *et al.*, 2014). Another explanation considers the effect of G-blocks on the electrostatic interactions within the mucus matrix. This scenario is exemplified by the observation of reduction in mechanical properties of *ex vivo* cystic fibrosis (CF) sputum after addition of G-block. Cystic fibrosis is characterized by thick mucus in several organs. This highly viscous mucus is especially problematic in the lungs, resulting in malfunction of the mucociliary clearance (Puchelle *et al.*, 2002). Electrostatic interactions between mucin and several non-mucin macromolecules present in CF mucus are suspected to increase the mucus mechanical

properties. A proposed mechanism for the observed effect of G-block involves interruption of the electrostatic interactions between mucins and macromolecules. G-blocks are oligoelectrolytes too small to make intermolecular cross-links. The idea is that the oligomers can disrupt and eliminate the interactions between mucins and macromolecules by electrostatic inhibition, thereby reducing the mechanical properties of mucus (Draget and Taylor, 2011).

1.1.6 RiXOVA

As described in section 1.1.2, one of the characteristics of solid tumours is changes in extracellular matrix. RiXOVA is an anti-cancer drug candidate developed by Professor Kurt I. Draget and Dr. Catherine T. Nordgård. It is based on G-block technology and targets ECM. The drug candidate is currently in the pre-clinical stage of testing in animal models. Experimental *in vivo* data has shown reduction in tumour growth in a mouse model of pancreatic cancer with RiXOVA both as a single agent and in combination with the standard cytostatic gemcitabine (Figure 1-5). However, the mode of action of RiXOVA remains to be understood. A hypothesis is that RiXOVA is able to reduce the extracellular matrix barrier to drug diffusion in tumours with an over-expression of ECM, such as pancreatic cancer, resulting in improved drug and immune system access to the cancer cells.

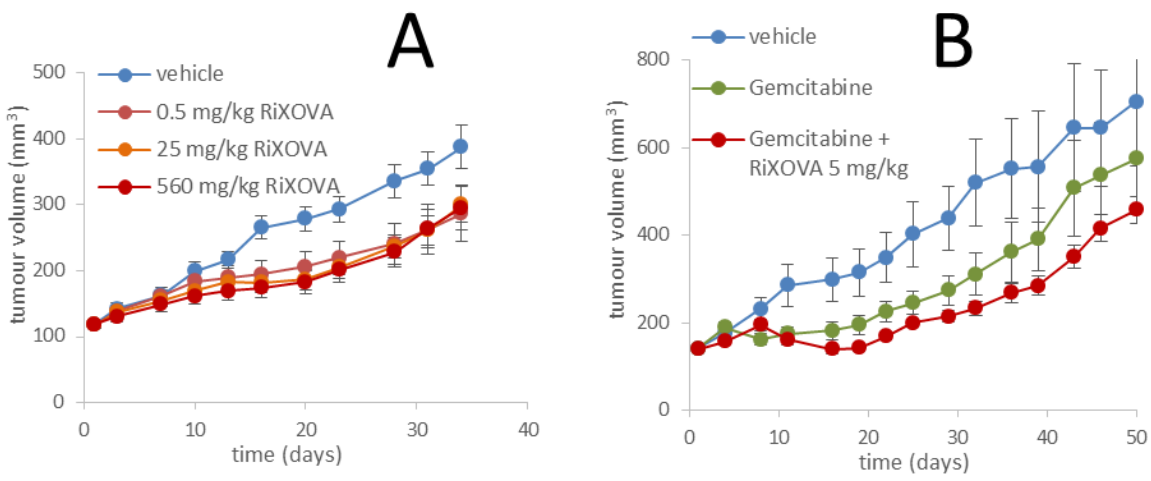


Figure 1-5. (A) Anti-tumour activity of RiXOVA and Gemcitabine *in vivo*, in Capan-2 human pancreatic tumour xenograft model. (B) Anti-tumour activity of RiXOVA as a single agent *in vivo*, in Capan-2 human pancreatic tumour xenograft model. Obtained by personal communication with Kurt I. Draget.

1.2 Aim of the thesis

The aim of this thesis is to investigate if G-block can reduce the extracellular matrix barrier to molecular diffusion. The first goal is to develop and optimize a method for studying diffusion of model compounds out of an extracellular matrix. Matrigel will serve as the model for ECM, whereas the model compounds to be tested include the dye tartrazine and Alexa Fluor® 488 IgG. Diffusion of the model compounds from Matrigel with and without G-block will be studied by utilizing the developed diffusion method to investigate whether G-block has an effect on molecular diffusion from Matrigel. Furthermore, the rheological profile of Matrigel with and without G-block is to be characterized to study the effect of G-block on the rheological properties of Matrigel.

1.3 Technical introduction

This section provides an introduction to the principles of the scientific techniques utilized in this thesis.

1.3.1 Spectrophotometry

Light can be considered as a collection of photons; particles carrying a fixed amount of energy. When a molecule absorbs a photon, the molecule is raised from its lowest energy state, the ground state, to an excited electron state. As the molecule return to its ground state, the energy absorbed is usually dissipated as heat. A molecule will only absorb photons of specific wavelengths corresponding to the absorption spectrum of the molecule. The maximum of this spectrum represents the wavelength of maximum electromagnetic absorption, λ_{max} (Harris, 2010). Molecules of different structure will absorb light from different parts of the electromagnetic spectrum, including the ultraviolet (UV) (190-400 nm) and visible (400-700 nm) region (Flanagan *et al.*, 2008).

Spectrophotometry is a technique utilizing the wavelength dependent absorbance of light by different molecules. The method provides quantitative measurements of light absorbed by a chemical compound and is often used to determine the concentration of an analyte in solution. Spectrophotometric measurements are carried out by using a spectrophotometer, which measures the intensity of light transmitted by a solution. A schematic diagram of a spectrophotometer is presented in Figure 1-6. Light is passed from a source through a monochromator, which is designed to select a narrow range of wavelengths to be send through the sample as monochromatic incident light of intensity I_0. The wavelength of the incident light is usually set to the absorbance maximum of the molecule of interest to obtain maximum sensitivity. The sample is kept in a transparent cuvette of a particular width. The analyte absorbs some of the incident light while the intensity of the transmitted light, I, is measured by a photodetector and recorded (Harris, 2010).

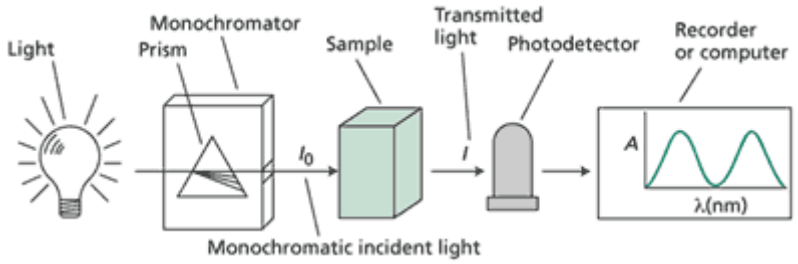


Figure 1-6. Schematic overview of the components of a spectrophotometer. Obtained from (Taiz and Zeiger)**.**

The absorbance, A, can be determined based on the transmittance. Transmittance, T, is defined as the fraction of incident light that is not absorbed by the chemical compound in the solution. The absorbance is defined in equation 1-2.

$$A = -\log T \qquad \textbf{(1-2)}$$

Furthermore, Beer's law (equation 1-3) states that, for a solution of an absorbing solute in a transparent solvent, the absorbance is directly proportional to the concentration of solute molecules.

$$A = \varepsilon bc \qquad \textbf{(1-3)}$$

where A is the dimensionless absorbance, c is the solute concentration (mol L^{-1}), b is the path length of the light through the cuvette (cm) and ε is the molar absorptivity (L cm^{-1} mol^{-1}). According to Beer's law, a plot of absorbance versus concentration will result in a straight line which can be used to determine the concentration of the absorbing molecule (Flanagan *et al.*, 2008).

1.3.2 Fluorescence spectroscopy

As mentioned in the previous section, absorbance of photons causes promotion of a molecule from the ground state to an excited electron configuration state. This is an energetically unfavorable and unstable state, favoring the molecule to return to its ground state after a short period of time. The absorbed energy is dissipated as heat and/or luminescence, depending on the molecular structure, the solvent, temperature and pressure. Luminescence is the emission of photons from a molecule in an excited state and can be divided into two categories; phosphorescence and fluorescence. The two types of luminescence are separated in terms of their lifetime, which is defined as the average time between a molecule's excitation and return to the ground state. The lifetime of phosphorescence is longer (10^{-4} to 10^{2} s) than the lifetime of fluorescence, which is typically in the range from 10^{-8} to 10^{-4} s (Harris, 2010, Flanagan *et al.*, 2008). The theory of fluorescence is explained with a Jablonski energy diagram in Figure 1-7.

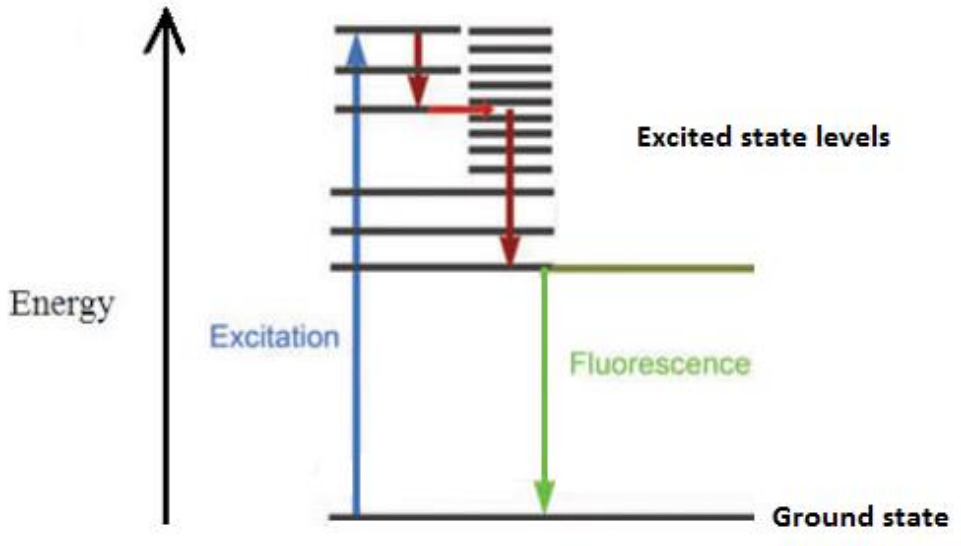


Figure 1-7. A simplified Jablonski energy diagram explaining the theory of fluorescence. An electron gains energy as it absorbs a photon, causing excitation of the molecule from the lower energy ground state to an unstable higher energy excited state. As the electron decay from the excited state after a short period of time, some of the absorbed energy is lost through radiationless transitions, such as molecular collisions. The rest of the energy is emitted as photons (fluorescence) before the molecule return to the ground state. Modified from (Lichtman and Conchello, 2005).

As illustrated in Figure 1-7, the energy of absorbed photons is generally higher than the energy of emitted photons, resulting in a longer wavelength of the emitted light compared to that of the absorbed light. This energy difference is referred to as the Stokes shift and occurs due to the dissipation of absorbed energy through radiationless transitions, such as molecular collisions and energy transfer between molecules (Lakowicz, 2007, Flanagan *et al.*, 2008). Chemical compounds emitting fluorescence are normally aromatic molecules, in which the part of the molecule responsible for the emission of fluorescence is named the fluorophore. The structure of the fluorophore determines the characteristic emission spectrum of a molecule, which is a plot of the fluorescence intensity versus emission wavelengths at a set excitation wavelength. Furthermore, the excitation spectrum is determined by monitoring the fluorescence emission against various excitation wavelengths at a constant emission wavelength (Lakowicz, 2007).

Fluorescence spectroscopy is a technique enabling high sensitivity detection of fluorescence emitted by a chemical compound. The relationship between the concentration of the fluorescent molecules in solution and the emitted fluorescence is linear for sufficiently dilute solutions, enabling concentration determination of the analyte in solution. The appropriate concentrations of solutions will vary among analytes, but is normally in the order of μg/ml. Higher concentrations will generally result in loss of the proportional relationship between fluorescence and concentration, often due to a phenomenon referred to as the inner-filter effect (Day and Underwood, 1991).

Fluorescence measurements are performed by a fluorometer, which measures the emitted fluorescence by a sample as a function of incident light (Lakowicz, 2007). The design of a conventional fluorometer is illustrated in Figure 1-8. Excitation incident light is passed from a source through a filter or monochromator to the sample, which is placed in a cuvette. Specific wavelengths of the incident light are absorbed by the analyte molecules, causing electron excitation and emission of fluorescence. The fluorescent light is passed through a second filter or monochromator before being measured by a detector and recorded. The detector is placed perpendicular to the incident light source to minimize the amount of incident light reaching the detector, thus reducing disturbance of fluorescence detection. The monochromators enables selection of the maximum excitation and emission wavelengths of the analyte, thus increasing the detection sensitivity (Flanagan *et al.*, 2008, Lakowicz, 2007).

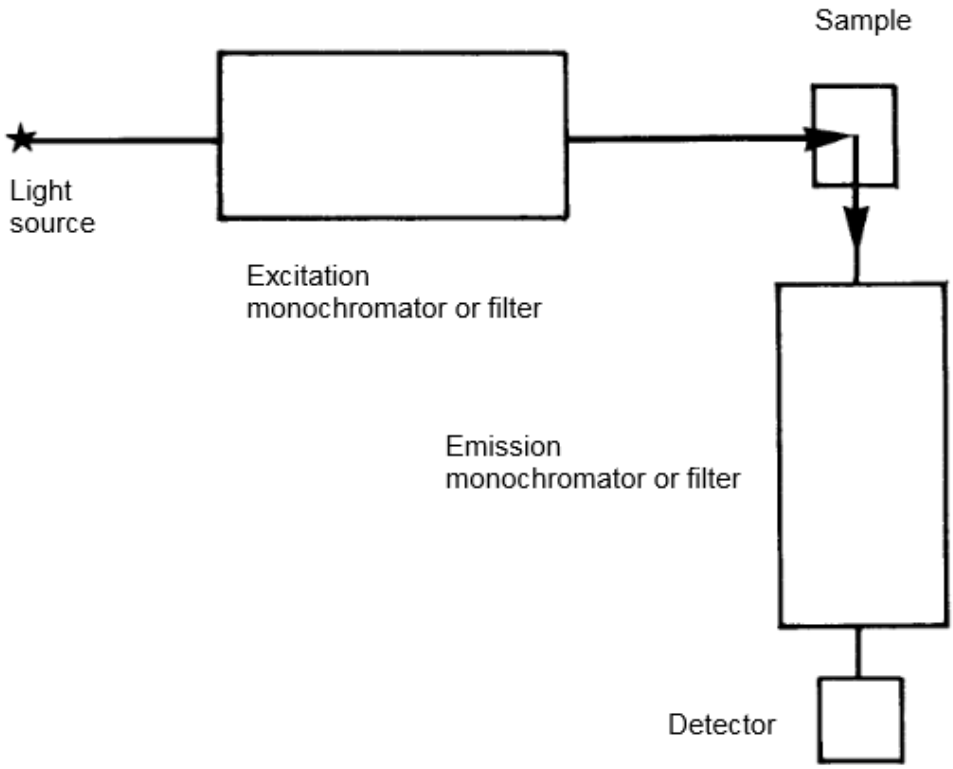


Figure 1-8. Basic design of a conventional fluorometer. Modified from (PerkinElmer, 2000).

1.3.3 Rheology

Rheology is the study of flow and deformation of materials, and provides information about the behaviour of a material in response to an applied force. All materials behave as solids or liquids, depending on the timescale of observation. Most solid materials flow as liquids at very long timescales under sustained stress, whereas liquids behave as solids at short timescales or high frequencies. Materials possessing both elastic and viscous properties at the same time are referred to as viscoelastic materials. The ratio of elastic and viscous properties of a material is determined by the duration of the rheological experiment (Picout and Ross-Murphy, 2003). Viscous properties are characterized by the loss of energy. As a force is applied to a viscous material, the energy used to deform the material is lost as heat and the material will persist in the new shape after removal of the force. Elastic properties are linked to the storage of energy. An elastic material will store the energy from the applied deformation and regain its original shape when the applied force is removed. The viscoelastic properties of a material can be determined by low deformation oscillatory rheological measurements (Smidsrød and Moe, 2008).

1.3.3.1 Rheological parameters

Processing rheological data involves several rheological variables. Important rheological parameters for this thesis are explained below.

Stress (τ) is defined as the force, F, acting per area, A. Stress has the unit Pascal (Pa) and produces a deformation (Picout and Ross-Murphy, 2003).

Strain (γ) or deformation is defined as the ratio of the change in dimension relative to the original dimensions as a result of applied stress. Strain is dimensionless (Picout and Ross-Murphy, 2003).

Shear strain rate ($\dot{\gamma}$) is the rate of change in shear strain with respect to time. The unit of shear strain rate is s^{-1} (Picout and Ross-Murphy, 2003).

The storage modulus (G') or elastic modulus is a measure of the elastic properties of a material. In general, the higher the values of G' is, the more solid-like behavior of the material. G' is measured in Pa (Smidsrød and Moe, 2008).

The loss modulus (G'') or viscous modulus characterizes the viscous properties of a material. In general, high values of G'' demonstrates a large proportion of viscous properties of the material. The unit of G'' is Pa (Smidsrød and Moe, 2008).

The complex modulus (G*) describes the ratio between the applied strain and the resultant stress, or vice versa (Picout and Ross-Murphy, 2003).

The phase angle (δ) is the phase difference between the applied strain wave and the resultant stress wave, or vice versa. The phase angle describes the relationship between the storage modulus (G') and the loss modulus (G'') (Picout and Ross-Murphy, 2003).

1.3.3.2 Oscillatory measurements

Oscillatory rheology enables quantification of the viscous and elastic properties of a material at various time scales (Wyss *et al.*, 2007). In small strain oscillatory measurements, rheological data can be obtained without destroying the structure of the material (Zhong and Daubert, 2013). Oscillatory measurements are performed by imposing oscillating shear strain on a sample and measure the resultant stress, or vice versa. The oscillating applied strain and the subsequent stress follow a sine wave, as illustrated in Figure 1-9. The amplitude of the waves corresponds to the applied strain and resultant stress, whereas the wavelength is determined by the frequency (Picout and Ross-Murphy, 2003).

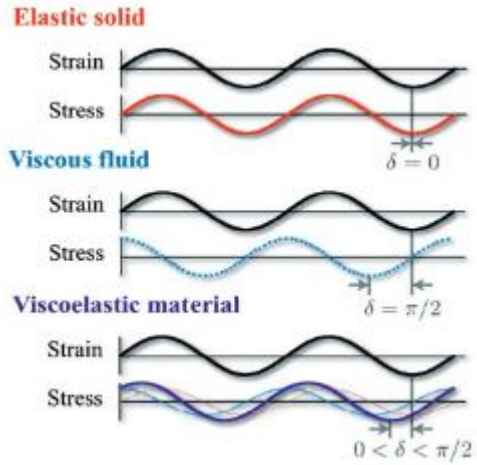


Figure 1-9. Stress response to applied oscillatory strain for an ideal elastic solid, an ideal viscous fluid and a viscoelastic material. Obtained from (Wyss *et al.*, 2007).

The stress response of a material depends on the proportion of elastic and viscous properties. An ideal elastic material will respond instantly to the imposed deformation, resulting in no phase lag between the applied strain and resultant stress. The stress wave will therefore be in phase with the strain wave, corresponding to a phase angle (δ) of 0º. For a perfect viscous material, the stress wave will be 90º out of phase with the strain wave (Picout and Ross-Murphy, 2003, Zhong and Daubert, 2013). The phase angle (δ) describes the relationship between the storage modulus (G') and the loss modulus (G'') (Picout and Ross-Murphy, 2003). G' quantifies the elastic properties of the material and is a measure of the ability of a material to recover its original shape (energy storage) after imposed deformation. G'' characterizes the viscous properties and is a measure of the energy loss of a material (Lai *et al.*, 2009). A viscoelastic material has a phase angle between that of an ideal elastic material and a perfect liquid (Zhong and Daubert, 2013). If $45º < \delta < 90º$, the loss modulus (G'') has greater values than the storage modulus (G') and the material will display more liquid-like, than solid-like, behaviour. If $0º < \delta < 45º$, the storage modulus (G') has greater values than the loss modulus and the material will show a more solid-like behaviour. The behaviour of a material is dependent on the frequency of the measurement, as the value of δ is only valid in the time scale of the measurements (Smidsrød and Moe, 2008).

The relationship between storage modulus (G'), loss modulus (G''), the phase angle (δ) and the complex modulus (G*) is illustrated in Figure 1-10 and described by equation 1-4 and 1-5 (Zhong and Daubert, 2013, Smidsrød and Moe, 2008).

$$\tan \delta = G''/G' \quad (1\text{-}4)$$

$$G^* = (G'^2 + G''^2)^{1/2} \quad (1\text{-}5)$$

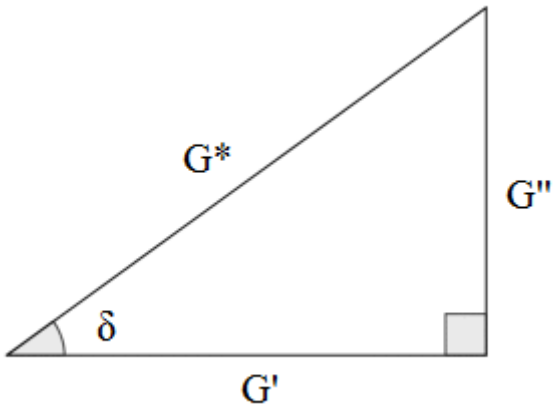
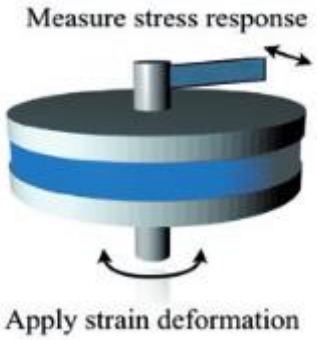
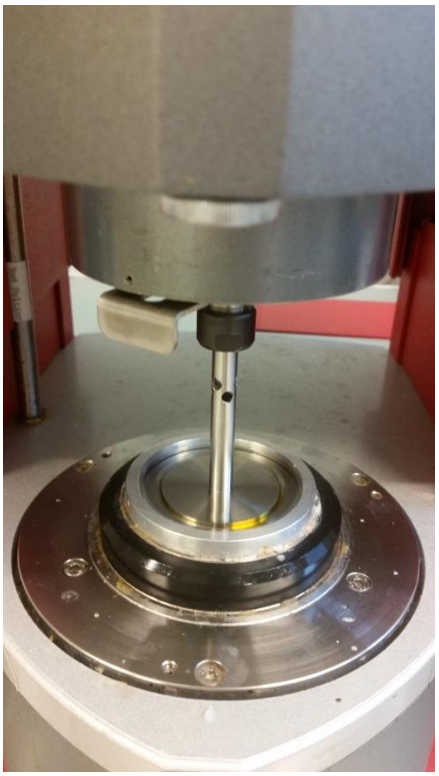


Figure 1-10. The relationship between the phase angle (δ), storage modulus (G'), loss modulus (G'') and complex modulus (G*).

Oscillatory rheological measurements are carried out by using a rheometer. The sample is loaded between two plates; a stationary plate and a rotating plate. A time dependent strain is applied to the sample as one of the plates rotate. At the same time, the resultant time dependent stress is quantified by measuring the torque imposed on the stationary plate by the sample. Both the strain and the strain rate (frequency) are controlled during measurements, enabling detection of differences in viscoelastic properties between various materials (Wyss *et al.*, 2007). Different geometries can be selected for the rheometer, including parallel plate and plate and cone geometry. The latter is utilized to provide a constant shear strain rate across the gap between the plate and the angled cone (Picout and Ross-Murphy, 2003). The experimental set-up for rheological measurements is illustrated in Figure 1-11.



a)



b)

Figure 1-11. a) Sample placed between a stationary plate and a rotating plate. b) Rheometer set-up with plate and cone geometry. The sample is loaded on the stationary plate and the cone, which oscillates during measurements, is lowered prior to measurements.

1.3.3.3 Strain sweeps

Strain sweeps are carried out to determine the linear viscoelastic region (LVR) and the stability of the material. Within this region, the moduli are independent of the applied strain and the deformation will not influence the properties of the material. To determine the LVR, a strain sweep is conducted by shearing the material in a strain range at a fixed frequency. Oscillation measurements are performed with a fixed strain within the LVR. A typical strain sweep result is presented in Figure 1-12. The plot can be divided into two regions: 1) the linear viscoelastic region characterized by a horizontal line and 2) a region displaying strain dependence. The two regions are separated by a strain value referred to as the limit of the linear viscoelastic region (γ_o) (Zhong and Daubert, 2013).

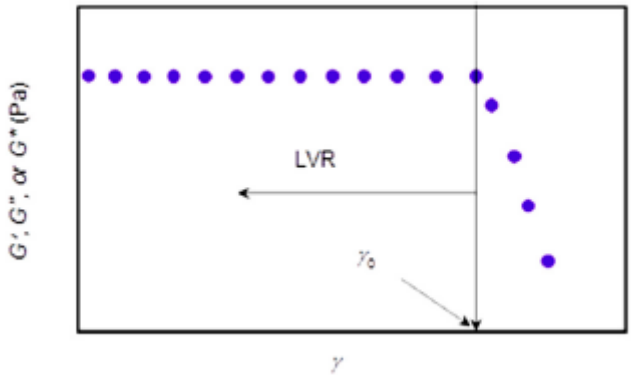


Figure 1-12. Typical strain sweep result used to determine the linear viscoelastic region (LVR) of a material. The moduli are independent on the applied strain in the LVR. Obtained from (Zhong and Daubert, 2013).

1.3.3.4 Frequency sweeps

Oscillation frequency is the reciprocal of the time it takes for the rheometer rotating plate to complete a sinusoidal oscillation cycle. Frequency sweeps are performed by applying a fixed oscillating strain within the LVR on a sample in a wide range of frequencies (Zhong and Daubert, 2013).

The frequency sweep describes the frequency dependence of the materials behaviour, also referred to as the mechanical spectrum. The behaviour observed in a frequency sweep is the bulk result of time-dependent entanglements, interactions and cross-links in the material (Picout and Ross-Murphy, 2003). The relationship between storage modulus (G') and loss modulus (G'') in a frequency sweep can determine whether the material can be categorized as a gel, concentrated solution or dilute solution (Zhong and Daubert, 2013). Characteristic mechanical spectra for a concentrated solution and a true gel system are presented in Figure 1-13. The concentrated solution is characterized by an entangled network as the concentration of polymer is so high that a uniform distribution of polymer segments among the solvent molecules occurs. At low frequencies, entanglement networks typically flow as high viscosity liquids with G'' > G'. As the frequency is increased, a "cross-over" between G' and G'' occurs and the network is showing gel system properties due to time-dependent interactions. True gel systems are normally independent of frequency and display no entanglement effects.

G’ and G’’ are parallel regardless of the frequency with G’ > G’’ in a gel system (Picout and Ross-Murphy, 2003).

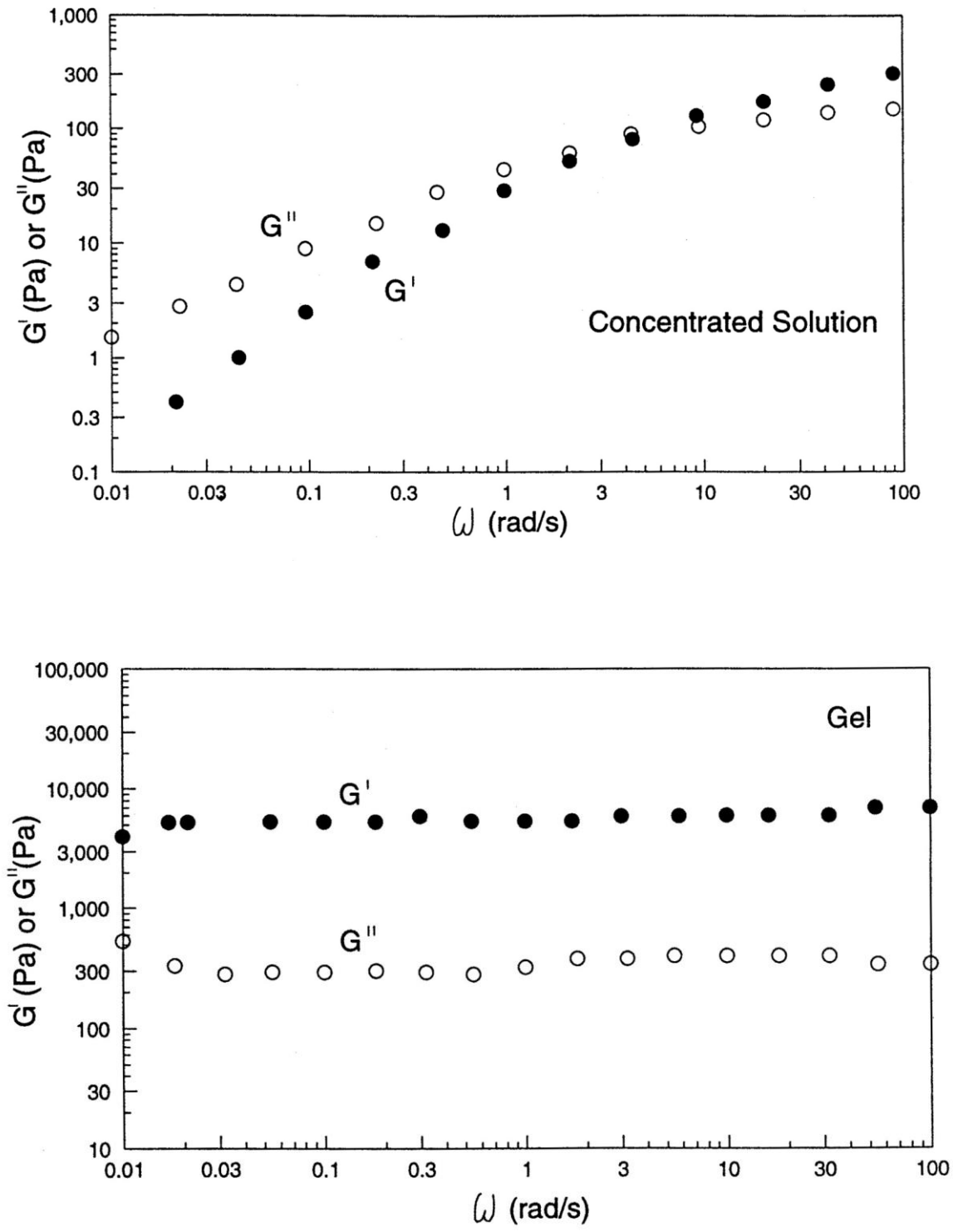


Figure 1-13. Typical mechanical spectra for a concentrated solution with an entangled network (top) and a true gel system (bottom). Adapted from (Ross-Murphy, 1984).

2. Materials and methods

2.1 Materials

2.1.1 Physiological saline solution

Physiological saline isotonic to tissue fluids (0.9 %) was prepared by dissolving sodium chloride (NaCl, 9 g) from Merck KGaA (Darmstadt, Germany, lot K44520304) in Milli-Q (MQ) water (1 L).

2.1.2 Corning® Matrigel® Basement Membrane Matrix

Corning® Matrigel® Basement Membrane Matrix purchased from Corning Inc. (Corning, New York, USA, catalog number 354234, lot 3340868) was utilized as a model for tumour extracellular matrix in this thesis. The provided Matrigel had a protein concentration of 8.4 mg/ml. Additional information can be found in the data sheet provided in Appendix A.

2.1.3 Tartrazine

Tartrazine from Sigma Aldrich Co. (St. Louis, USA, lot 079K1462V) was used as the initial model compound in the study of molecular diffusion. Stock solutions were prepared by dissolving tartrazine in physiological saline (NaCl, 0.9 %). Saline was used as solvent in all dilutions of tartrazine stock solutions.

2.1.4 Alexa Fluor® 488 goat anti-human IgG (H+L)

Alexa Fluor® 488 goat anti-human IgG (H+L) (2 mg/ml) purchased from Life Technologies (Paisley, Scotland, lot 1495793) was utilized as a model compound to investigate diffusion of macromolecules. Alexa Fluor® 488 IgG is an antibody conjugated to the green-fluorescent dye Alexa Fluor® 488, which has an excitation maximum of 490 nm and an emission maximum of 525 nm (Life Technologies(A)). Alexa Fluor® 488 IgG is labelled with 7 moles of dye per mole of protein, enabling detection by fluorescence spectroscopy (Life Technologies(C)). Physiological saline was used as solvent in dilutions of the provided Alexa Fluor® 488 IgG stock solution.

2.1.5 Alginate G-block

The alginate G-block utilized in this thesis was a G-block DP 12 sample prepared by Camilla Reehorst 8th of September 2013. The alginate G-block sample was produced by acid hydrolysis of "G-blokk H3" provided by FMC Biopolymer AS (Drammen, Norway). The number-average degree of polymerization (DP_n) was determined to be 12 by applying ^1H NMR spectroscopy, which also revealed that the fractions of guluronate containing monad (F_G), diad (F_{GG}) and triad (F_{GGG}) were 0.93, 0.83 and 0.81, respectively (Reehorst, 2014).

Isotonic G-block stock solution (70 mg/ml) was prepared by dissolving G-block (DP 12) in MQ water, followed by mixing to form a homogenous solution. Further dilutions were made by using saline as solvent. The G-block solutions were stored in a refrigerator.

2.1.6 Transwell® Permeable Support

The diffusion experiments were carried out in Transwell® Permeable Support well plates purchased from Corning Inc. (Corning, New York, USA). The Transwell plates contained two separate units; (1) the top Transwell filter insert and (2) the bottom lower Transwell compartment. The Transwell inserts had a diameter of 6.5 mm. The membrane material was polycarbonate with pore sizes of 0.4 μm and nominal pore density 1 x 10^8 pores/cm^2. Recommended volume added to the lower compartment was 0.6 ml, whereas recommended volume added to the inside of Transwell insert was 0.1 ml (Corning(B)).

2.2 Methods

2.2.1 Diffusion experiment

2.2.1.1 Experimental design

The objective of developing a diffusion experiment method was to establish a model enabling study of diffusion of molecules, including macromolecules, through extracellular matrix. Development of such a method was crucial for being able to compare molecular diffusion through ECM with and without added G-block. The diffusion of molecules through extracellular matrix was investigated using the yellow dye tartrazine as the initial model compound and Matrigel as a model for extracellular matrix. Tartrazine was later replaced by the antibody Alexa Fluor® 488 goat anti-human IgG (H+L) in order to study diffusion of macromolecules from Matrigel with and without G-block.

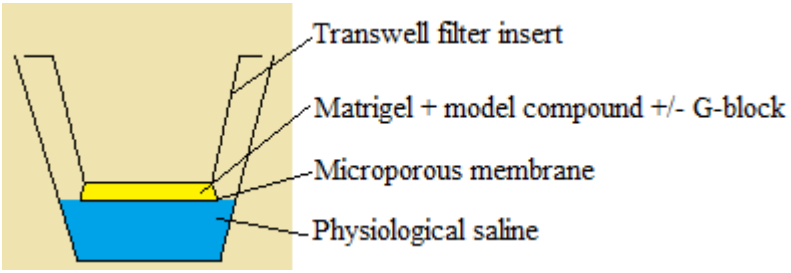


Figure 2-1. Experimental set-up for diffusion of model compounds through extracellular matrix (Matrigel).

The experimental set-up is illustrated in Figure 2-1. A gel consisting of Matrigel, model compound (tartrazine or Alexa Fluor® 488 IgG) and physiological saline (NaCl, 0.9 %) or G-block was placed in a Transwell insert and positioned above and in contact with a solution of physiological saline in the well. Model compounds were incorporated in the gel and not placed on top of the gel to avoid additional error sources. Placing the compounds on top of the gel would require a uniform and intact gel surface to achieve comparable diffusion in all experiments. The model compound was expected to diffuse down its concentration gradient from the Matrigel into the saline solution. Diffusion of model compound into the receiving chamber was monitored over time by removing samples from the saline solution at different times during the experiment. A volume of saline corresponding to the removed sample

volume was added to the well after each sampling to maintain contact between the Transwell insert and the solution in the well. The concentration of model compound in each sample was determined by spectrophotometry or fluorescence spectroscopy, depending on the type of model compound used in the experiment.

2.2.1.2 Basic protocol

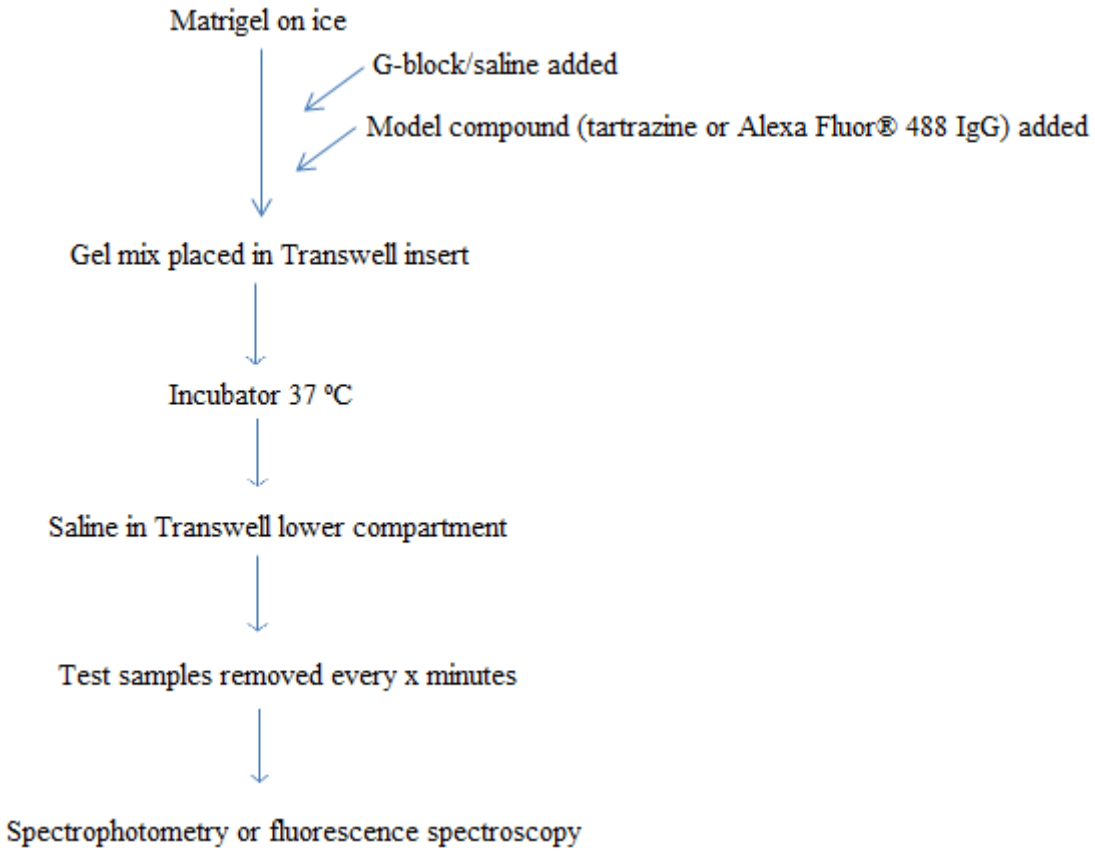


Figure 2-2. Schematic overview of the basic steps of the diffusion experiment.

Figure 2-2 presents a flowchart of the fundamental steps in the experimental design of the diffusion experiment. This basic protocol served as a framework for further optimization of the method.

2.2.1.3 Protocol optimization

Optimization of the basic protocol into a final protocol was crucial in the development of the method for studying molecular diffusion from Matrigel. The process included several steps of optimization, which is described below.

Determining gel volume in Transwell insert

The amount of gel mixture added to the Transwell insert was determined prior to the start of the diffusion experiments. The main requirement was that the gel covered the Transwell membrane filter. The recommended volume added to the inside of Transwell insert was 0.1 ml according to the Transwell protocol (Corning(B)). However, in order to reduce the amount of material used, it was investigated if the gel volume could be reduced and still cover the Transwell membrane. The required gel volume was determined by adding volumes of water less than 0.1 ml to the insert followed by observation of the coverage of the filter. Volumes from 60 µl to 100 µl were observed to completely cover the Transwell filter. To allow for some margin of error in the practical performance of the production of gels, the required gel volume in the Transwell insert was determined to be 80 µl.

Determining saline volume in Transwell chamber

The volume of saline in the Transwell chamber was determined to be the recommended volume of 600 µl according to the Transwell protocol (Corning(B)) to establish contact between the Transwell insert and the saline solution.

Determining sample volume

The sample volume removed from the saline solution in the Transwell lower compartment during the diffusion experiment was determined by adding the recommended volume of 600 µl water to well chambers followed by removal of various test volumes. Dilution steps described in section 2.2.1.4 Final protocol; Diffusion experiment procedure contributed to low concentrations of the model compounds throughout the diffusion experiment. Therefore, it was desirable to use a sample volume as large as possible to accomplish minimum dilution and end up with a detectable model compound concentration in the cuvette. However, practical hurdles limited the maximum sample volume to 300 µl as it was challenging to remove larger volumes without pipetting air due to the short distance between the bottom of

the chamber and the surface of the water. The sample volume was therefore determined to be 300 µl.

Determining cuvette sample volume

There has to be a certain sample volume in a cuvette to detect absorbance or fluorescence when performing spectrophotometry or fluorescence spectroscopy. The required sample volume is related to the position of the light source or laser and the type of cuvette utilized. During the diffusion experiment, samples of 300 µl were removed, placed in cuvettes and diluted with saline to reach the required volume of detection. It was desirable to achieve minimum dilution of the sample in the cuvette as the model compound concentration already was low before the final step of dilution. The minimum cuvette sample volume was determined by filling a cuvette with a model compound solution of known concentration, followed by monitoring the absorbance/fluorescence of the sample as the volume in the cuvette was reduced. The minimum sample volume was reached when the measurements became unstable and deviated from the known values. Based on the results, the required cuvette sample volumes were determined to be 1800 µl and 1400 µl for the two types of cuvettes utilized in spectrophotometric measurements. Cuvettes used in fluorescence spectroscopy were determined to have a required sample volume of 2000 µl.

Absorbance spectrum of tartrazine

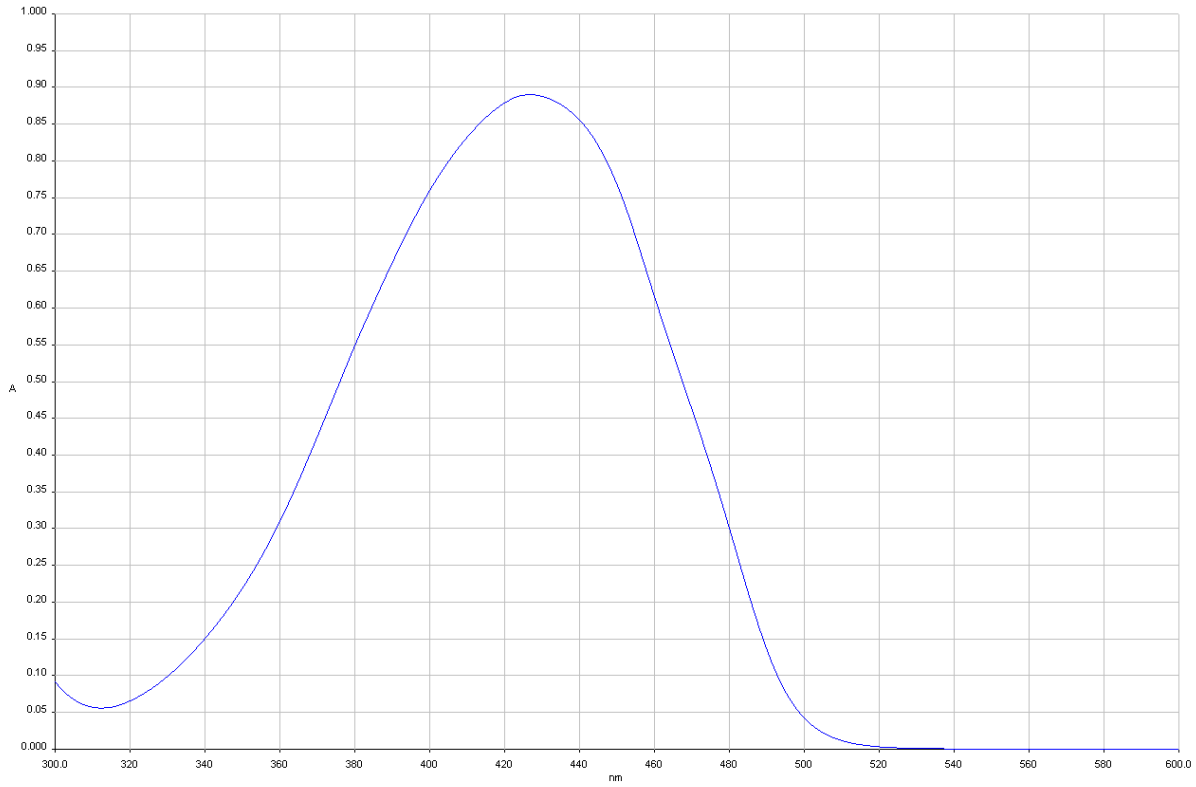


Figure 2-3. Absorbance spectrum of tartrazine. The absorbance is plotted against the wavelength. The wavelength of maximum absorbance (λ_{max}) of tartrazine was determined to be 426 nm.

The absorbance spectrum of tartrazine is presented in Figure 2-3. The spectrum was used to determine the wavelength of maximum absorbance (λ_{max}) of tartrazine. It was important to determine λ_{max} as this value is characteristic for each compound. Furthermore, λ_{max} was utilized in spectrophotometric measurements of samples containing tartrazine. The spectrum was prepared by measuring the absorbance of light in the wavelength range of 300 to 600 nm of a sample of tartrazine (0.0377 mM) with measured maximum absorbance of 0.8946. This particular sample was chosen because the maximum absorbance was just below 1, resulting in a steep curve enabling determination of λ_{max}. Samples with absorbance above 1 will exceed the linear area in which the ratio between concentration and absorbance is linear according to Beer's law (Flanagan *et al.*, 2008). λ_{max} of tartrazine was determined to be 426 nm according to the peak of the spectrum.

Determining concentration of tartrazine in Matrigel

Another step in the development and optimization of the method for studying diffusion of tartrazine from Matrigel was to find the optimal concentration of tartrazine in the gel. There are several steps of dilution in the diffusion experiment. Therefore, it was crucial to find a tartrazine concentration in the gel resulting in detectable concentrations of tartrazine in the final saline solution in the cuvette at all times of sampling during the experiment. At the same time, it was also important to find a concentration in which the diffusion rate was optimal for practical purposes. An excessive diffusion rate would lead to limited information over a short period of time and result in poor basis for comparison. In contrast, a too low diffusion rate would be impractical as the duration of the experiment would be long. An optimal tartrazine concentration in the gel would result in a tartrazine diffusion rate enabling the experiment to be performed during a work day with detectable tartrazine concentration in each sample throughout the experiment. The tartrazine concentration in the saline solution at each time of sampling is also influenced by the frequency of sampling. Therefore, both frequency of sampling and the optimal tartrazine concentration in Matrigel was optimized at the same time. The optimization of sampling frequency is described in the next section.

The approach to determine the concentration of tartrazine in the gel was to first find the range of tartrazine concentrations corresponding to absorbance 1 and down to the minimum detectable concentration of the spectrophotometer. Secondly, a tartrazine concentration within this measurable range was tested by performing a diffusion experiment and analyzing the results. A standard curve of tartrazine was prepared by measuring the absorbance of a set of standard tartrazine solutions by spectrophotometry at wavelength $\lambda = 426$ nm. Solutions with concentrations corresponding to absorbance values between 1 and 0, and consequently within the area in which the ratio between absorbance and concentration is linear, were used in the preparation of the standard curve. Measured absorbance was plotted against known concentrations, producing the standard curve presented in Figure 2-4.

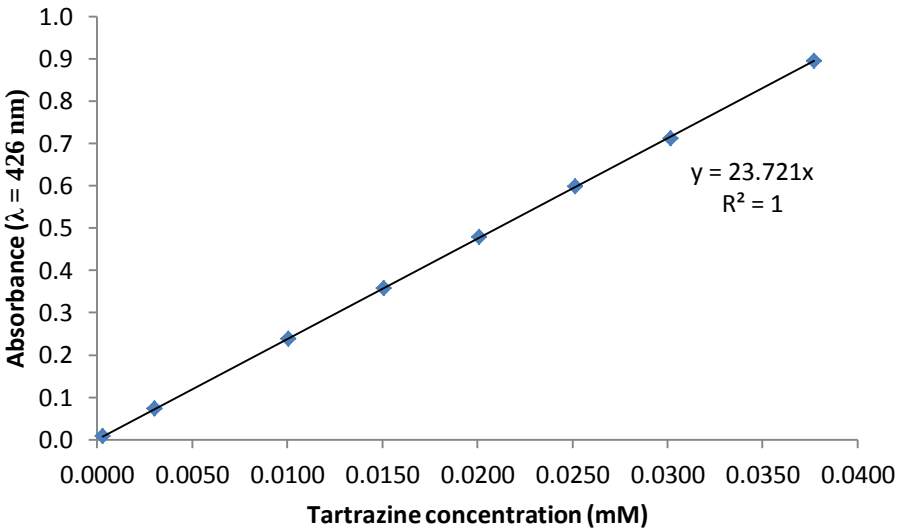


Figure 2-4. Tartrazine standard curve. Absorbance of tartrazine standard solutions was plotted against known concentrations. Measurements were performed at wavelength 426 nm.

The highest and lowest tartrazine concentration in the standard curve was 0.03771 mM and 0.00030 mM with absorbance value 0.8946 and 0.0084, respectively. The equation of the trend line intersecting the points was determined to be y = 23.721x. The coefficient of determination (R^2) equaled 1, indicating that the points fitted the straight trend line perfectly.

The upper limit of tartrazine concentration possible in the final saline solution in the cuvette after all dilution steps in the diffusion experiment was determined to be 0.03771 mM, corresponding to the highest concentration in the standard curve. The highest possible tartrazine concentration in the gel was calculated to be 1.70 mM based on a cuvette sample volume of 1800 µl, sample volume of 300 µl and gel volume of 80 µl. The calculations can be found in Appendix B.1.

An additional standard curve with tartrazine solutions of concentrations corresponding to absorbance values ranging from 0.114 to 0.0005 were used to find the minimum detection limit of the spectrophotometer in terms of the lowest tartrazine concentration the instrument is able to detect. The standard curve with the lower tartrazine concentrations plotted against absorbance of the respective concentrations is presented in Figure 2-5.

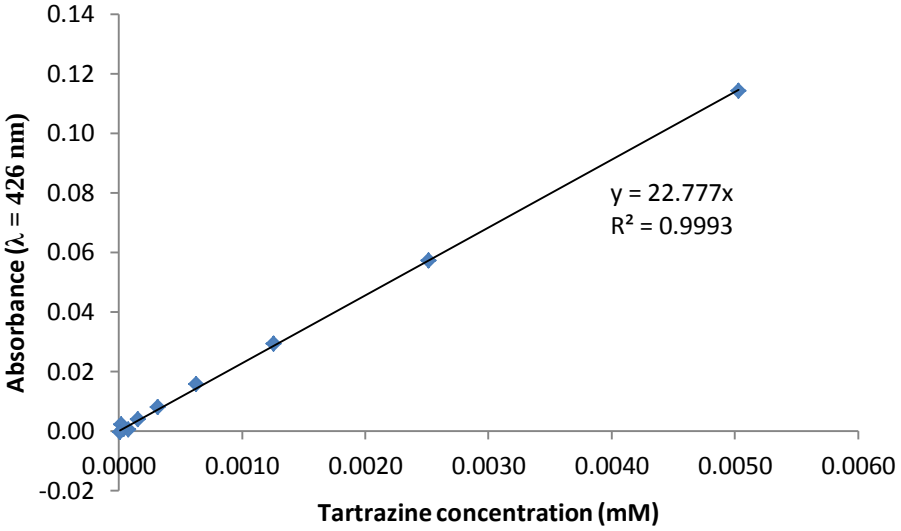


Figure 2-5. Standard curve of tartrazine. Absorbance of tartrazine standard solutions was plotted against known concentrations. Measurements were performed at wavelength 426 nm.

The minimum tartrazine concentration detected by the spectrophotometer was determined to be 0.000314 mM as lower concentrations resulted in unstable absorbance measurements and values close to or below 0. In addition, values below this limit did not fit the trend line and were therefore not in accordance with the equation of the standard curve. The equation of the trend line intersecting the points was determined to be y = 22.777x. R^2 equaled 0.9993, indicating that the points fitted the straight trend line.

The lower limit of tartrazine concentration possible in the final saline solution after all dilution steps in the diffusion experiment was determined to be the minimum detected concentration times ten (0.00314 mM) to allow some margin of error. The lowest tartrazine concentration possible to use in the gel was calculated to be 0.14 mM based on a cuvette sample volume of 1800 µl, sample volume of 300 µl and gel volume of 80 µl. The calculations can be found in Appendix B.1.

As a result of the standard curves in Figure 2-4 and Figure 2-5, the maximum and minimum measurable tartrazine concentrations were set to be 0.0377 mM and 0.000314 mM, respectively. Absorbance of concentrations between these extreme points was within the linear region of the standard curve and could be measured by the spectrophotometer. Data points from both standard curves presented were assembled into a final standard curve, presented in Figure 2-6. The points exceeding the determined limits of maximum and minimum measurable tartrazine concentrations were excluded from the final standard curve.

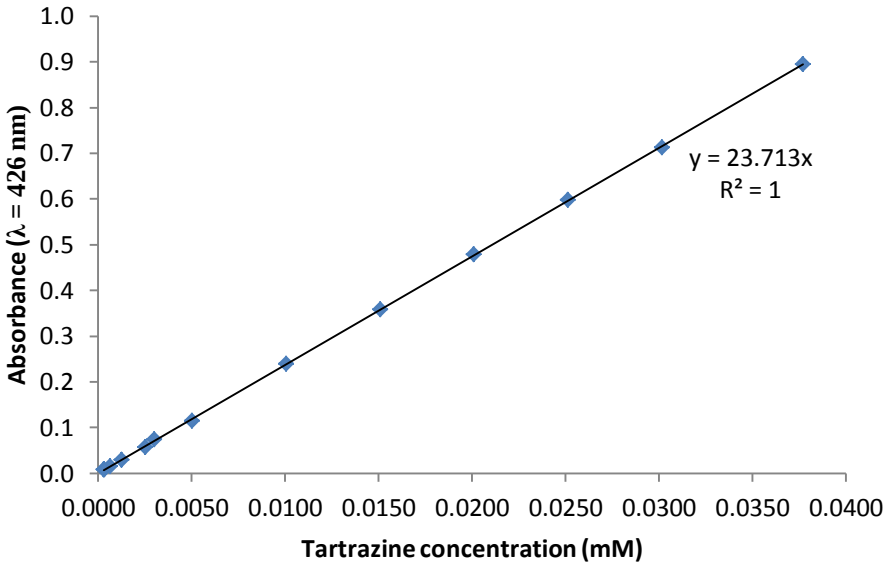


Figure 2-6. Final tartrazine standard curve. Absorbance of standard solutions plotted against known concentrations. Measurements were performed at wavelength 426 nm.

The equation of the trend line intersecting the points was determined to be y = 23.713x. R^2 equaled 1, indicating that the points fitted the straight trend line perfectly.

As mentioned earlier, the calculated maximum and minimum tartrazine concentrations in Matrigel were 1.70 mM and 0.14 mM, respectively. Based on these limits, the first tested gel concentration of tartrazine was chosen to be 1 mM. The concentration was tested by carrying out a test diffusion experiment with tartrazine (1 mM) in Matrigel, followed by analysis of the diffusion rate of tartrazine. The same test experiment was also used for determining frequency of sampling and the duration of the experiment assuming a correlation between tartrazine concentration in Matrigel, tartrazine diffusion rate and sampling frequency. Further details of the procedure of this experiment are presented in the next section. The results supported the test concentration of 1 mM to be an optimal tartrazine concentration in Matrigel as the diffusion rate resulted in sample tartrazine concentrations within the measurable range. Results for sampling frequency and duration are further discussed in the next section.

Determining frequency of sampling and duration of diffusion experiment

A test diffusion experiment with tartrazine (1 mM) in Matrigel was performed in order to determine the optimal frequency of sampling and the duration of the experiment. 5 replicates were made and gels were prepared according to section 2.2.1.4 Final protocol; preparation of gels. However, no magnetic stirrer was used. The diffusion experiment was performed as described in 2.2.1.4 Final protocol; diffusion experiment procedure, although frequency of sampling and duration were not determined at this point. The first Transwell insert was loaded into the chamber containing saline, directly followed by loading of the other replicate inserts. The first sample of all replicates was removed after 5 minutes and the following samples were removed every 15^{th} minute during a time period of 155 minutes. From 155 minutes and up to 275 minutes, sampling was carried out every 30^{th} minute due to the assumption that most of tartrazine in the gel would diffuse out early in the experiment. Sampling from replicate wells was performed without a set time interval, but immediately after one another and in the same order at every time of sampling. The resulting diffusion curve is displayed in Figure 2-7. Calculation example is presented in Appendix C.1 and raw data can be found in Appendix C.2.

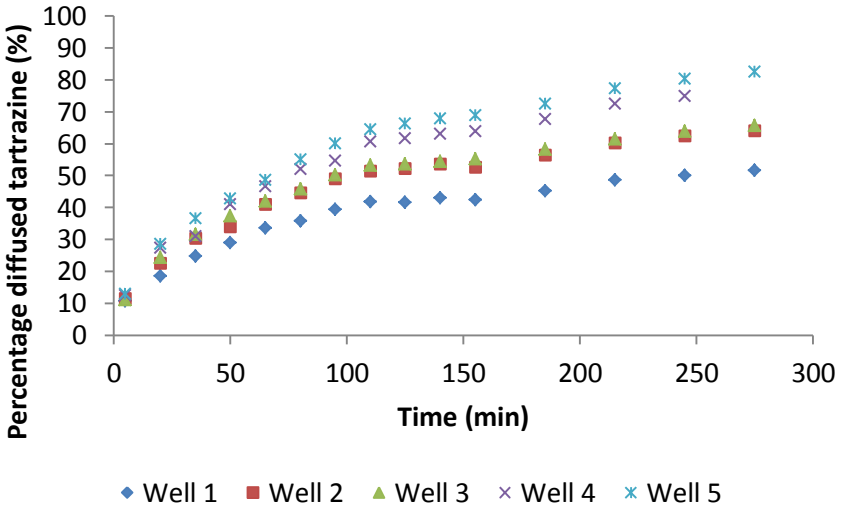


Figure 2-7. Tartrazine diffusion curve. The diffusion experiment was performed with tartrazine (1 mM) in Matrigel for 275 minutes.

The amount of tartrazine diffused from Matrigel was distributed according to the well order of sampling. In addition, the standard deviation at each point of sampling increased throughout the experiment and it was a large variation of total diffused tartrazine between replicates after 275 minutes. It was also observed that a sampling frequency of every 15th minute was excessive and impractical. However, the experiment duration of 4.5 hours and the test tartrazine concentration of 1 mM resulted in measurable sample concentrations throughout the experiment and a diffusion curve giving sufficient information for comparison.

In addition to the samples collected in the initial phase of the experiment, sampling after 24 hours was conducted in order to study the maximal amount of model compound able to diffuse from Matrigel. The results of the 24 hours sample could also locate possible interactions between model compounds and Matrigel, as some molecules might be trapped inside the gel network due to interactions between model compound molecules and components of the Matrigel.

Another test diffusion experiment with tartrazine (1 mM) in Matrigel was conducted with some procedure changes. Use of a magnetic stirrer was incorporated into the preparation of gels procedure to obtain a homogenous solution. Transwell replicate inserts were loaded into their respective chambers with a time gap of 1 minute. Frequency of sampling was reduced to every 30th minute during a time period of 270 minutes in addition to introducing a time interval of 1 minute between sampling in each replicate. The diffusion curve is presented in Figure 2-8. Raw data can be found in Appendix C.2.

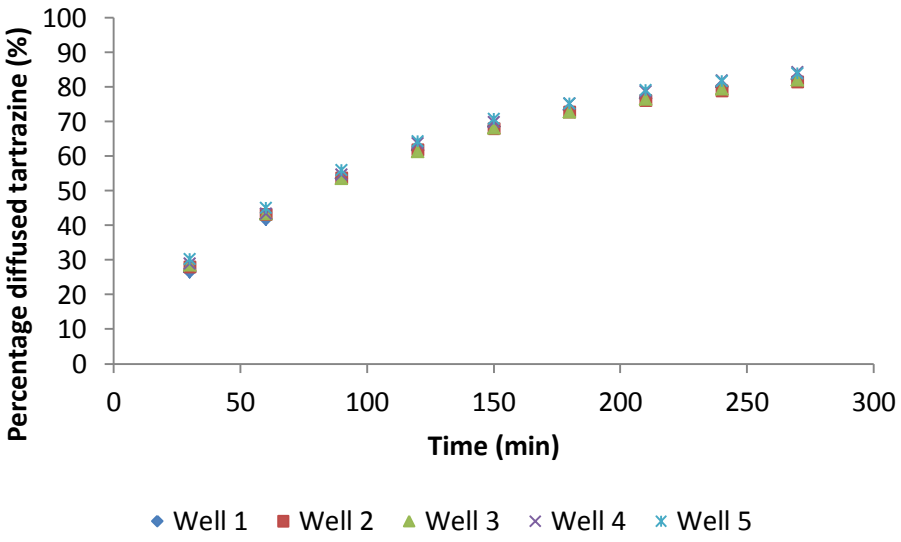


Figure 2-8. Tartrazine diffusion curve after optimization. The diffusion experiment was performed with tartrazine (1 mM) in Matrigel for 270 minutes. Sampling was conducted every 30th minute.

The combination of the optimized tartrazine concentration (1 mM) and the procedure changes contributed to reduced standard deviations at each point of sampling, higher average amount of total diffused tartrazine after 270 minutes and no result distribution according to well sample order. The optimal frequency of sampling was therefore determined to every 30th minute for 4.5 hours in addition to a 24 hour sample. A time interval of 1 minute between loading and sampling in replicates was also incorporated in the diffusion experiment procedure.

Determining start concentration of G-block in Matrigel

The start concentration of G-block in Matrigel was determined based on the dosing of G-block in a completed *in vivo* study in a mouse model of pancreatic cancer (section 1.1.6). In this study, the mice received an intravenous dose corresponding to a theoretical G-block blood concentration of 0.4 mg/ml. Due to pharmacokinetics such as distribution and metabolism it is reasonable to assume that less than 0.4 mg/ml reached the final target tissue of the pancreatic tumour. However, since the amount of G-block at the target site was unknown, the initial concentration of G-block in Matrigel was based on the theoretical blood concentration and was determined to be 0.5 mg/ml.

Determining concentration of Alexa Fluor® 488 IgG in Matrigel

A standard curve was prepared in order determine the concentration of Alexa Fluor® 488 IgG in Matrigel. Fluorescence of Alexa Fluor® 488 IgG standard solutions was measured by fluorescence spectroscopy. Concentrations with fluorescence within the linear region of the standard curve were included in the standard curve, which is presented in Figure B.3.1 in Appendix B.3. An optimal concentration of IgG in Matrigel would result in sample fluorescence values within the linear area of the standard curve, enabling calculation of the concentration of diffused IgG from Matrigel in each sample. It would be most practical to use the provided stock solution concentration to avoid light exposure when making dilutions. Furthermore, it would also be practical to use the same volume of IgG as tartrazine (75 µl) in other gel mixtures. It was calculated that if 75 µl of the provided stock solution (2 mg/ml) was used, the concentration in Matrigel would be 0.25 mg/ml and the maximum diffused Alexa Fluor® 488 IgG concentration in one sample (assuming all of IgG to diffuse at once) would be 0.0333 mg/ml, which was within the linear region of the standard curve. It was further assumed that the lowest sample fluorescence values of IgG during the diffusion experiment would be within the detectable range of the luminescence spectrometer. The results from the first diffusion experiment with Alexa Fluor® 488 IgG in Matrigel supported the concentration of 0.25 mg/ml to be optimal, as IgG concentrations within the linear area of the standard curve were obtained at each sampling point (section 3.1.7, Figure 3-9).

2.2.1.4 Final protocol

Preparation of Matrigel gels

Matrigel was thawed on ice in a 4 ºC refrigerator overnight. Preparation of gel mixtures was carried out in the refrigerator with all material on ice at all times to avoid gelling of Matrigel. Depending on the experiment, either saline or G-block solution (DP 12) was added to the vial containing Matrigel, followed by mixing with a magnetic stirrer to form a homogenous solution. Either tartrazine or Alexa Fluor® 488 IgG was added to the solution before mixing. Alexa Fluor® 488 IgG solution was mixed before addition. The composition of the different gel mixtures utilized in this thesis is presented in Table 2-1. Each gel mixture also contained Matrigel (450 µl) in addition to the content displayed in the table. Gel mixture (80 µl) was added to the bottom of a Transwell insert which was loaded into the Transwell chamber in a 24 well plate with lid (Figure 2-9 and 2-10). The Transwell plate was placed at 37 ºC for 30 minutes to allow setting of the gel. The handling of Matrigel was performed according to the manufacturer's protocol, which can be found in Appendix A. Gels containing Alexa Fluor® 488 IgG were covered with aluminum foil after gel setting and during the experiment in order to prevent light exposure.

Table 2-1. Overview of the composition of gel mixtures prepared for diffusion experiments. All gel mixtures also contained 450 µl of Matrigel. The total volume of each gel mixture was 600 µl.

Gel	Composition			
	Saline	Tartrazine (conc. in gel)	G-block DP 12 (conc. in gel)	Alexa IgG (conc. in gel)
A1	75 µl	75 µl, 8 mM (1 mM)		
A2		75 µl, 8 mM (1 mM)	75 µl, 4 mg/ml (0.5 mg/ml)	
A3		75 µl, 8 mM (1 mM)	75 µl, 40 mg/ml (5 mg/ml)	
A4		75 µl, 8 mM (1 mM)	75 µl, 0.4 mg/ml (0.05 mg/ml)	
B1	75 µl	75 µl, 4 mM (0.5 mM)		
B2		75 µl, 4 mM (0.5 mM)	75 µl, 40 mg/ml (5 mg/ml)	
C1	75 µl	75 µl, 2 mM (0.25 mM)		
C2		75 µl, 2 mM (0.25 mM)	75 µl, 40 mg/ml (5 mg/ml)	
D1	150 µl			
D2	75 µl		75 µl, 40 mg/ml (5 mg/ml)	
E1	75 µl			75 µl, 2 mg/ml (0.25 mg/ml)
E2			75 µl, 40 mg/ml (5 mg/ml)	75 µl, 2 mg/ml (0.25 mg/ml)
F1	135 µl			15 µl, 2 mg/ml (0.05 mg/ml)
F2	60 µl		75 µl, 40 mg/ml (5 mg/ml)	15 µl, 2 mg/ml (0.05 mg/ml)

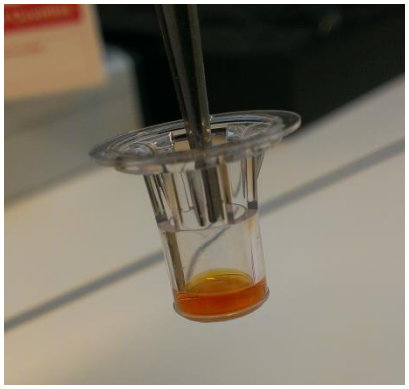


Figure 2-9. Transwell insert with gel containing Matrigel, saline and tartrazine.

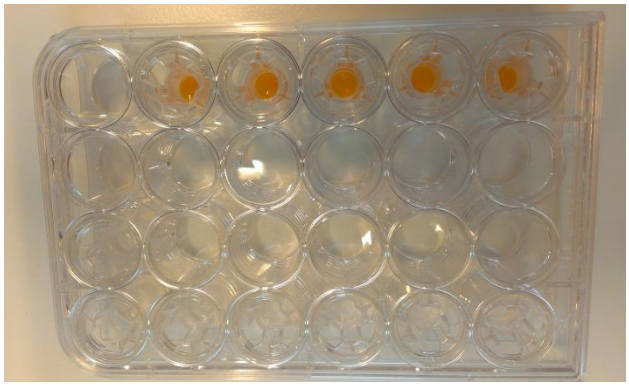


Figure 2-10. Transwell plate containing five replicates of Transwell inserts with gels consisting of Matrigel, saline and tartrazine.

Diffusion experiment procedure

The diffusion experiment was conducted by loading the Transwell insert with gel into the Transwell chamber containing a solution of physiological saline (600 µl). Replicates were loaded with a set time interval of 1 minute. Sampling was performed every 30th minute for 4.5 hours by transferring the Transwell insert with a tweezer to an adjacent empty well and transferring a sample (300 µl) from the saline solution into a cuvette. Saline (300 µl) was added to the Transwell before the insert was loaded back into the chamber. Both the duration of each sampling and the time interval between sampling for each replicate was 1 minute. The Transwell plates were covered with lid between sampling. In addition to the samples collected in the first 4.5 hours of the experiment, sampling after 24 hours was conducted in order to study the total amount of diffused model compound from Matrigel. Samples in the cuvettes were diluted with saline until the required sample volume was reached; 1800 µl and 1400 µl for spectrophotometric measurements and 2000 µl for fluorescence measurements. The cuvettes were covered with parafilm and stored in a box until the end of the experiment.

Absorbance measurements

Absorbance measurements of samples from diffusion experiments with gels containing tartrazine were performed using a Lambda 25 UV/VIS Spectrometer (Perkin Elmer Instruments, Massachusetts, USA) at wavelength 426 nm. Saline was used as a blank to subtract background absorbance. Components of Matrigel and G-block were assumed not to contribute to any background absorbance of importance. Absorbance values were displayed in the software UV WinLab and further analyzed in Microsoft Excel. The tartrazine concentration in each sample was calculated by using the equation of the standard curve in Figure 2-6 in section 2.2.1.3 Protocol optimization; Determining concentration of tartrazine in Matrigel. An additional standard curve was made due to switch of cuvette type and can be found in Appendix B.2, Figure B.2.1.

Fluorescence measurements

Fluorescence measurements of samples from diffusion experiments with gels containing Alexa Fluor® 488 IgG were carried out utilizing a Luminescence Spectrometer LS 50 B (Perkin Elmer Instruments, Massachusetts, USA) with a set excitation maximum of 490 nm and emission maximum of 525 nm. Control diffusion experiments without Alexa Fluor® 488 IgG were performed to investigate the amount of background fluorescence from Matrigel components and G-block. Fluorescence values were obtained from the software FL WinLab and analyzed in Microsoft Excel. Concentration of Alexa Fluor® 488 IgG in each sample was determined by using the equation of the standard curve in Figure B.3.1 in Appendix B.3.

2.2.1.5 Statistical analyses

Statistical analyses were carried out on the diffused model compound concentration values for each time of sampling to determine whether there was a significant difference of diffusion from Matrigel with and without G-block. It was also investigated whether experiments with Matrigel containing G-block displayed significantly higher diffusion rate than Matrigel without G-block.

Differences were examined by a two sample T-test with assumed unequal variances in Microsoft Excel. The T-test compares two population means to determine if they are significantly different. The confidence interval was set to 95 % in all statistical analyses assuming statistical significance when the p-value was less than 0.05.

Below are some assumptions made when performing a two sample T-test.

H_0: Population 1 = Population 2

H_1: Population 1 ≠ Population 2 H_1: Population 1 > Population 2

P > 0.05: H_0 is accepted, H_1 is rejected → concentration value 1 = concentration value 2

P < 0.05: H_1 is accepted, H_0 is rejected → concentration value 1 ≠ concentration value 2

→ concentration value 1 > concentration value 2

2.2.2 Rheological measurements

All rheological measurements were run on a Rheologica StressTech rheometer (Rheologica, Lund, Sweden, serial number 212-0752) with cone and plate geometry. The cone used in all measurements had a diameter of 40 mm and an angle of 1 degree. Prior to measurements the cone was lowered to "zero gap" and approximately 350 µl of sample was added to the plate, which was chilled to 4 ºC. The sample was then covered in low viscosity silicone oil (Dow Corning ® 200/10cS fluid, VNR International LTD, Butterworth, UK, lot 0805006) to avoid evaporation of water from the samples during measurement. The measurements were registered in the software Rheoexplorer and analyzed in Microsoft Excel.

Table 2-2 displays an overview of the content of the different gel mixtures prepared for characterization by rheological measurements. The mixtures were prepared according to the procedure described in section 2.2.1.4 Final protocol, preparation of gels. However, the mixtures were directly applied to the plate of the rheometer after mixing of all components instead of incubation.

Table 2-2. Overview of the composition of gel mixtures prepared for rheological measurements.

Gel	Composition			
	Matrigel	Saline	G-block DP10 (conc. in gel)	Tartrazine (conc. in gel)
1	450 µl			
2	450 µl	150 µl		
3	450 µl	75 µl	75 µl, 40 mg/ml (5 mg/ml)	
4	450 µl	75 µl		75 µl, 8 mM (1 mM)
5	450 µl		75 µl, 40 mg/ml (5 mg/ml)	75 µl, 8 mM (1 mM)

2.2.2.1 Oscillation strain control

Oscillation strain control measurements of Matrigel were first conducted by increasing the temperature gradually from 4 ºC to 37 ºC over a period of 35 minutes. All other measurements were run after a quick ramp of the temperature from 4 ºC to 37 ºC prior to measurements. All measurements were run at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz.

2.2.2.2 Frequency sweeps

Frequency sweeps were run directly after oscillation strain control on all samples with a fixed strain of $5*10^{-3}$. Measurements were performed in the interval $5*10^{-3}$-10 Hz, which was later reduced to 0.02-10 Hz.

2.2.2.3 Strain sweeps

Frequency sweeps were immediately followed by a strain sweep in the strain region $1*10^{-4}$-1 to determine the viscoelastic region. The strain interval was later increased to $1*10^{-4}$-100 to study the material during destruction. All strain sweeps were conducted with a fixed frequency of 1 Hz. The set strain of $5*10^{-3}$ used in the frequency sweeps were compared to the linear viscoelastic region determined by the strain sweeps to confirm that the applied strain was within this region.

3. Results and discussion

3.1 Diffusion experiments

In order to test the hypothesis that G-block can reduce the extracellular matrix barrier to drug diffusion in tumours with an over-expression of ECM, it was crucial to establish a method for studying diffusion of model compounds. After several steps of development and optimization described in section 2.2.1 Diffusion experiment, a diffusion experiment enabling comparison of molecular diffusion from Matrigel with and without G-block was established. The diffusion experiment method was utilized to investigate the diffusion of tartrazine and IgG from Matrigel.

3.1.1 Tartrazine diffusion curve

Several steps of optimization of the diffusion experiment method (see section 2.2.1 Diffusion experiment) resulted in the tartrazine diffusion curve presented in Figure 3-1. The curve is a result of a diffusion experiment with Matrigel containing tartrazine (1 mM) conducted according to section 2.2.1.4 Final protocol. Sampling was performed every 30^{th} minute for 4.5 hours in 5 replicates, in addition to a 24 hour sample. The concentration of tartrazine in each sample was determined by absorbance measurements and the standard curve presented in Figure 2-6, section 2.2.1.3 Protocol optimization; Determining concentration of tartrazine in Matrigel**.** Raw data can be found in Appendix C.2. The concentration of diffused tartrazine from Matrigel was further calculated by including all dilution steps of the method. Furthermore, the percentage diffused tartrazine at each sampling point was calculated as a percentage of the maximum potential concentration of diffused tartrazine in the saline solution. This value was calculated based on the assumption that all tartrazine in Matrigel diffuses into the saline solution in order to simplify the calculations. However, it is assumed that a fraction of tartrazine will remain in the gel. A concentration of 1 mM tartrazine in the gel corresponded to a maximum potential diffused tartrazine concentration of 0.133 mM in the saline solution. Calculation examples can be found in Appendix C.1. The same calculation method was used in all diffusion experiments with tartrazine and IgG as model compounds. The percentage diffused tartrazine after 270 minutes and ≈ 24 hours is presented in Table 3-1.

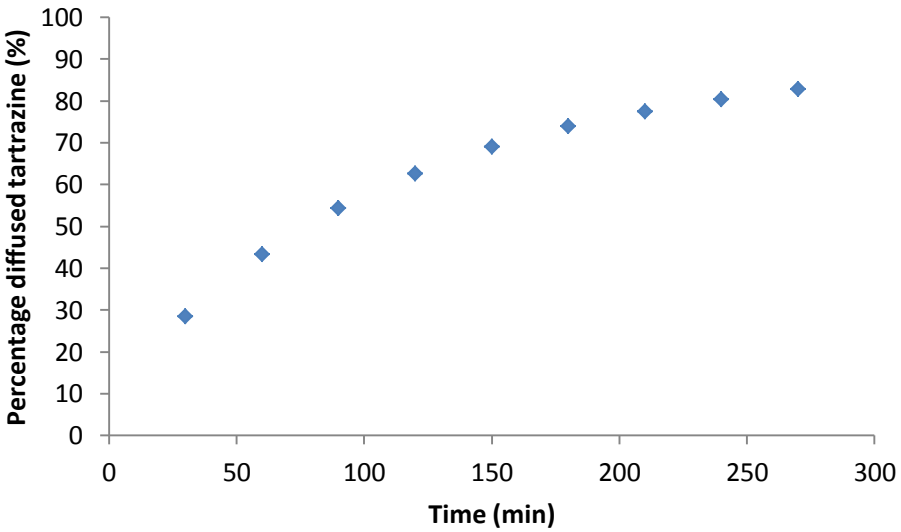


Figure 3-1. Tartrazine diffusion curve as a result of a diffusion experiment with tartrazine (1 mM) in Matrigel. Each point represents the mean percentage diffused tartrazine of 5 replicates. Typical standard deviations were ±1.2 %.

Table 3-1. Percentage diffused tartrazine of maximum (0.133 mM in saline solution) after 270 minutes and ≈ 24 hours. Results are given as mean percentage ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	82.9 ± 1.2	91.2 ± 1.2

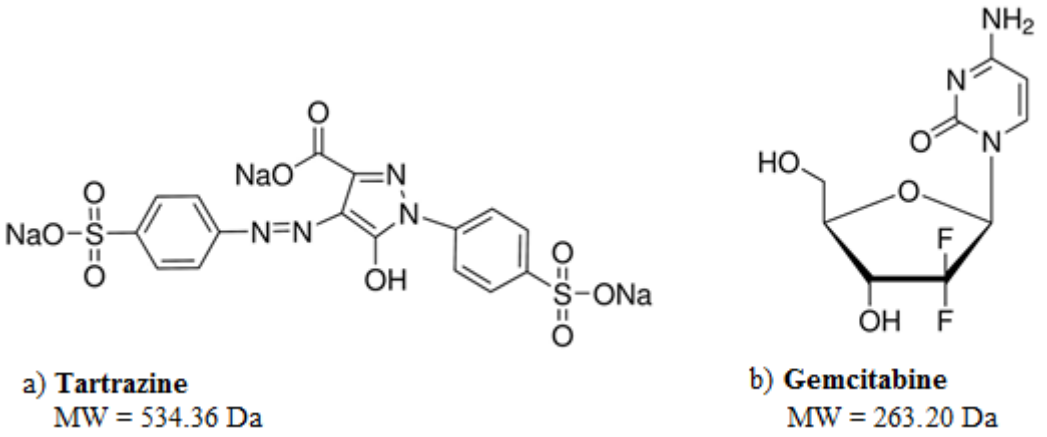
As demonstrated in Figure 3-1, tartrazine is diffusing down its concentration gradient from Matrigel into the saline solution, resulting in an increased concentration of tartrazine in the saline solution. Tartrazine is a hydrophilic molecule expected to diffuse rapidly into aqueous solutions like the saline solution in the Transwell. The diffusion rate is highest in the two first hours before it flattens out. The percentages displayed in Table 3-1 demonstrate that tartrazine diffusion is rapid in the first hours of the experiment with 83 % of tartrazine diffused from Matrigel after 270 minutes. From this point and up to 24 hours the diffusion is leveling off with an increase of 8 % of diffused tartrazine in the course of 19.5 hours, leaving a fraction of 9 % of tartrazine left in the Matrigel after 24 hours.

The difference in diffusion rate throughout the experiment might be explained by a combination of two theories. The first theory is that the change in diffusion rate might be caused by the decrease of the tartrazine concentration gradient as tartrazine diffuses from Matrigel into the saline solution. The concentration gradient is to some extent maintained throughout the experiment by removal of tartrazine from the saline solution during sampling. However, tartrazine will accumulate in the saline solution at the same time as the concentration of tartrazine in Matrigel is reduced as tartrazine diffuses into the saline solution. The second explanation of the transition from higher to lower diffusion rate in the tartrazine diffusion curve considers the possible interactions between tartrazine and Matrigel. The components of a gel may affect diffusion by binding the solute (Muhr and Blanshard, 1982). Therefore, it is a possibility that components of Matrigel interacts with a fraction of tartrazine, thus obstructing or delaying the diffusion of bound tartrazine from Matrigel into the saline solution. The interactions may reach a level of saturation and the excess tartrazine will be free and diffuse more rapidly from the Matrigel. The combination of rapid diffusion of free tartrazine and the more slowly diffusion of bound tartrazine may result in a diffusion curve similar to that of Figure 3-1.

The results were verified by performing an identical, independent diffusion experiment with tartrazine (1 mM) in Matrigel. Raw data can be found in Appendix C.2. Statistical analyses supported the verification as there was no significant difference between the diffusion rates of the two experiments at any sampling points (CI = 95 %, $p>0.05$). Data from the statistical analysis are presented in Appendix D. Due to the reproducible results, this diffusion experiment served as a control experiment for experiments with Matrigel containing G-block in addition to tartrazine (1 mM).

Tartrazine served as the initial model compound in this project because of its hydrophilic nature, small size and non-hazardous properties. Tartrazine is a synthetic yellow azo dye used in food colouring as E number E102 (de Andrade *et al.*, 2014). It was assumed that tartrazine would diffuse from Matrigel relatively rapid due to its high water-solubility and low molecular weight. The small size of tartrazine resembles the size of the standard-of-care chemotherapeutic drug gemcitabine, which was used in combination with G-block in a mouse *in vivo* pancreatic cancer model described earlier in the introduction. The structure and molecular weight of tartrazine and gemcitabine are presented in Figure 3-2. Size similarity is

beneficial when constructing a model system for studying diffusion, because the rate and degree of molecular diffusion is dependent on the radius of the molecules according to the Stokes-Einstein equation (see section 1.1.4 Diffusion, equation 1-1). It was also advantageous to use a dye because the concentration in solution could be determined easily by spectrophotometry.



a) **Tartrazine**
MW = 534.36 Da

b) **Gemcitabine**
MW = 263.20 Da

Figure 3-2. Structure and molecular weight of a) the yellow azo dye tartrazine and b) the cytostatic gemcitabine used in cancer treatment (Sigma-Aldrich(A), Sigma-Aldrich(B)).

3.1.2 The effect of G-block on diffusion of tartrazine from Matrigel

In order to test the effect of G-block on diffusion of molecules from Matrigel, a diffusion experiment with Matrigel containing tartrazine (1 mM) and G-block (0.5 mg/ml, DP 12) was performed according to section 2.2.1.4 Final protocol. 5 gel replicates were made and sampling was performed every 30th minute for 4 hours instead of 4.5 hours due to practical issues with the timekeeping at the final sampling. The concentration of tartrazine in each sample was determined by absorbance measurements and the standard curve presented in Figure 2-6, section 2.2.1.3 Protocol optimization; Determining concentration of tartrazine in Matrigel**.** The resulting tartrazine diffusion curve is presented in Figure 3-3, in which control experiment data (see section 3.1.1 Tartrazine diffusion curve) is also included. Raw data is given in Appendix C.2. Sampling after 24 hours was also conducted. Percentage diffused tartrazine at each sampling point was calculated based on the maximum potential concentration of diffused tartrazine in the saline solution. A concentration of 1 mM tartrazine in the gel corresponded to a maximum potential diffused tartrazine concentration of 0.133 mM in the saline solution. The percentage diffused tartrazine after 240 minutes and ≈ 24 hours is presented in Table 3-2.

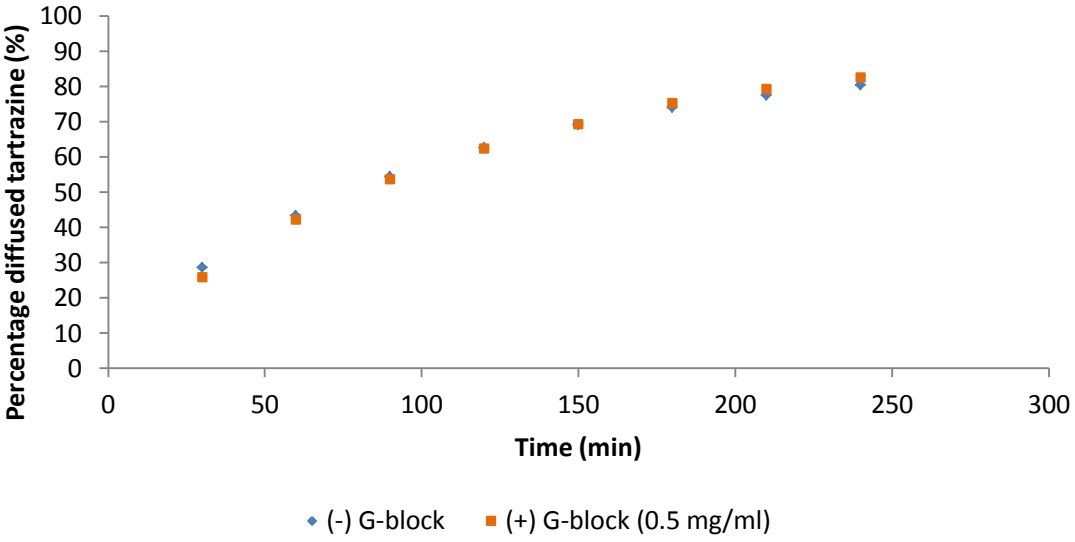


Figure 3-3. Tartrazine diffusion curve for diffusion experiment with G-block (0.5 mg/ml, DP 12) and control (without G-block). Each point represents the mean percentage diffused tartrazine of 5 replicates. Typical standard deviations were in the range from ±0.2 % to ±1.9 %.

Table 3-2. Percentage diffused tartrazine of maximum (0.133 mM in saline solution) after 240 minutes and ≈ 24 hours. Results are given as mean percentage ± standard deviation.

	240 min (%)	≈ 24 hours (%)
Control	80.5 ± 1.3	91.2 ± 1.2
(+) G-block (0.5 mg/ml)	82.7 ± 0.4	95.3 ± 0.7

Figure 3-3 demonstrates a significant higher tartrazine diffusion rate in the control compared to the experiment with G-block (0.5 mg/ml) at the first sampling point after 30 minutes (CI = 95 %, $p<0.05$). From this point and up to 210 minutes, there is no significant difference in diffusion rate between the experiment and the control (CI = 95 %, $p>0.05$). However, the experiment with G-block (0.5 mg/ml) displays a significant higher diffusion rate than the control at the sampling points at 240 minutes and 24 hours (CI = 95 %, $p<0.05$). Results from the statistical analyses are summarized in Table 3-3. Further information from the statistical analyses can be found in Appendix D. In addition, the amount of tartrazine left in the Matrigel after 24 hours was reduced from 9 % in the control to 5 % in the experiment with G-block (0.5 mg/ml).

Table 3-3. Statistical analyses of tartrazine diffusion in diffusion experiments with G-block concentration 0.5 mg/ml compared to control. Results are presented as p-values with significant difference labelled with *. CI = 95 %.

Sampling time (min)	p-value
30	0.038* (control higher)
60	0.087
90	0.274
120	0.744
150	0.703
180	0.068
210	0.051
240	0.018*
≈ 1440 (24 hours)	0.001*

Although there is a significant higher tartrazine diffusion rate from Matrigel containing G-block at the last sampling point after 240 minutes and the 24 hour sample, the magnitude of the difference can be questioned. The difference in diffusion rate between the control and the G-block experiment is only 2 % and 4 % at 240 minutes and 24 hours, respectively (Table 3-2). Based on these results, there is not enough information or data supporting an effect of G-block on tartrazine diffusion from Matrigel.

The network of Matrigel is a complex system with several components, resulting in various possible interactions with tartrazine and G-block. A factor possibly contributing to the lack of effect of G-block on tartrazine diffusion in Matrigel might be the addition order of tartrazine and G-block in the preparation of the gels. In this diffusion experiment, tartrazine was added before G-block, which may promote interactions between tartrazine and components of Matrigel before addition of G-block. This may prevent or delay potential interactions between G-block and Matrigel. Therefore, it can be questioned whether G-block (0.5 mg/ml) would have had a larger effect on tartrazine diffusion if the order of addition was different. Due to the possible importance of addition order, it was decided to change the order and add G-block before tartrazine to Matrigel in the following experiments.

Based on the discussed results, it was decided to investigate the diffusion of tartrazine from Matrigel in a wider range of G-block concentrations. The next step was therefore to perform two additional diffusion experiments with a tenfold increased and decreased G-block concentration, respectively.

3.1.3 Increasing/decreasing G-block concentration by a ten-fold

To investigate the effect of G-block on tartrazine diffusion from Matrigel in a larger range of G-block concentrations, two additional diffusion experiments were carried out according to section 2.2.1.4 Final protocol with Matrigel containing tartrazine (1 mM) and; (1) G-block (5 mg/ml, DP 12) and (2) G-block (0.05 mg/ml, DP 12). The order of addition to Matrigel was changed to G-block being added before tartrazine in the preparation of gels. The number of gel replicates was 4 and 5 for experiment 1 and 2, respectively. The replicate number deviated due to some practical issues with sampling in the 5^{th} replicate of experiment 1. Sampling was conducted every 30^{th} minute for a period of 4.5 hours in both experiments, in addition to a 24 hour sample. The concentration of tartrazine in each sample was determined by absorbance measurements and the standard curve presented in Figure 2-6, section 2.2.1.3 Protocol optimization; Determining concentration of tartrazine in Matrigel**.** Figure 3-4 displays the tartrazine diffusion curve for both experiments, in addition to the control experiment described in section 3.1.1 Tartrazine diffusion curve. Raw data is given in Appendix C.2. Percentage diffused tartrazine at each sampling point was calculated based on the maximum potential concentration of diffused tartrazine in the saline solution. A concentration of 1 mM tartrazine in the gel corresponded to a maximum potential diffused tartrazine concentration of 0.133 mM in the saline solution. The percentage diffused tartrazine after 270 minutes and ≈ 24 hours is presented in Table 3-4.

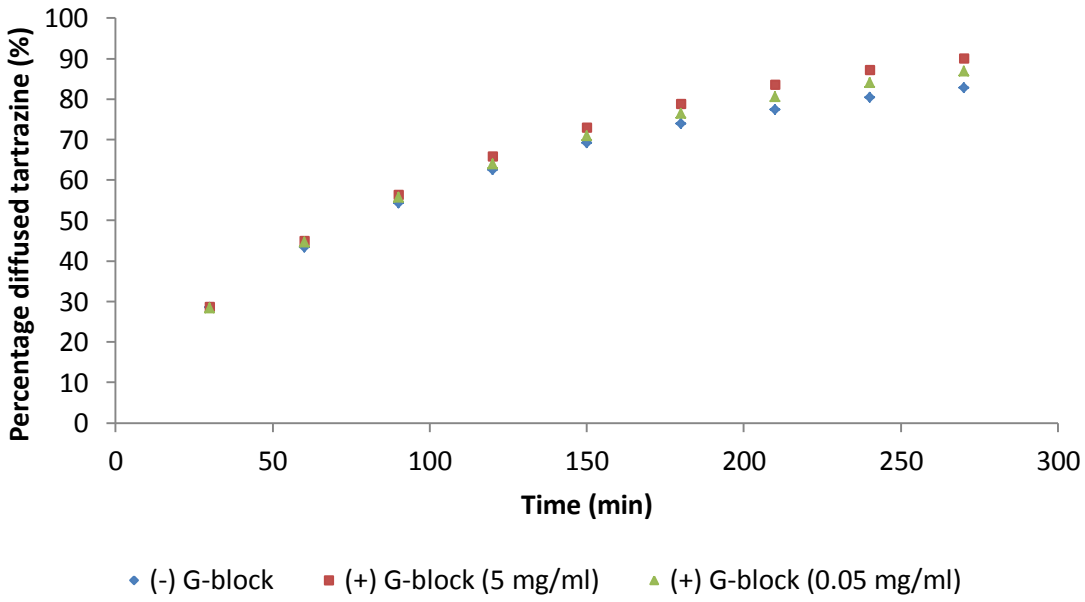


Figure 3-4. Tartrazine diffusion curve for diffusion experiments with tartrazine (1 mM) and; (1) G-block (0.05 mg/ml, DP 12) and (2) G-block (5 mg/ml, DP 12) in addition to the control experiment (without G-block). Each point is given as the mean percentage diffused tartrazine of the replicates. Typical standard deviations for all curves were in the range from ±0.3 % to ±1.7 %.

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Table 3-4. Percentage diffused tartrazine of maximum (0.133 mM in saline solution) after 270 minutes and ≈ 24 hours. Results are presented as mean percentage ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	82.9 ± 1.2	91.2 ± 1.2
(+) G-block (0.05 mg/ml)	87.0 ± 0.5	94.9 ± 0.9
(+) G-block (5 mg/ml)	90.1 ± 0.8	98.0 ± 0.4

Figure 3-4 and Table 3-4 demonstrates a significant higher tartrazine diffusion rate in the diffusion experiment with G-block (0.05 mg/ml) compared to the control experiment at each sampling, including the 24 hour sample, (CI = 95 %, $p<0.05$) except from the sampling points at 30, 60 and 120 minutes (CI = 95 %, $p>0.05$). The diffusion rate of tartrazine in the diffusion experiment with G-block (5 mg/ml) is observed to be significantly higher than the control at each sampling point and the 24 hour sample (CI = 95 %, $p<0.05$), except from the samples collected at 30 and 60 minutes (CI = 95 %, $p>0.05$). In addition, comparison of the two experiments with G-block (5 mg/ml) and G-block (0.05 mg/ml) demonstrates a

significantly higher diffusion rate in Matrigel with G-block (5 mg/ml) from 120 minutes and up to the 24 hour sample (CI = 95 %, $p<0.05$). Results from the statistical analyses are summarized in Table 3-5. Further details can be found in Appendix D.

Table 3-5. Statistical analyses of tartrazine diffusion in diffusion experiments with G-block concentration 0.05 mg/ml and 5 mg/ml compared to control. The experiments with G-block were also compared. Results are presented as p-values with significant difference labelled with *. CI = 95 %.

	p-values		
Sampling time (min)	**0.05 mg/ml**	**5 mg/ml**	**5 mg/ml > 0.05 mg/ml**
30	0.828	0.840	0.660
60	0.087	0.172	0.790
90	0.032*	0.031*	0.341
120	0.083	0.002*	0.010*
150	0.023*	0.001*	0.010*
180	0.011*	0.000*	0.0046*
210	0.009*	0.000*	0.002*
240	0.003*	0.000*	0.001*
270	0.000*	0.000*	0.001*
≈ 1440 (24 hours)	0.001*	0.000*	0.001*

As demonstrated in Table 3-5, a significant difference in tartrazine diffusion between the control and both experiments with G-block is observed from the sampling point at 90 minutes and up to 24 hours. The similar diffusion rate of all experiments in the first 60 minutes might be caused by diffusion of a free tartrazine fraction that is not affected by G-block. It is reasonable to assume that the observed difference in tartrazine diffusion after 90 minutes is caused by G-block, as G-block is the only component differing from the control experiment.

The mechanism of how G-block interferes with Matrigel and tartrazine to increase the diffusion rate can be a complex process with several possible interactions involved. A hypothesis is that G-block affects the structure of the Matrigel network, resulting in reduced steric hindrance and higher diffusion rate of the model compound. However, steric hindrance is not likely to be of importance with tartrazine as model compound, as tartrazine is a small molecule.

Another hypothesis is that the observed increase in tartrazine diffusion rate is partly caused by competitive binding of G-block and tartrazine to Matrigel. Binding of G-block to Matrigel will then cause a reduction in the bound fraction of tartrazine, leading to an increased fraction of free tartrazine that will diffuse more rapidly into the saline solution. As a result, the total diffusion at each sampling point will be higher than without G-block present. The hypothesis is supported by the results from the diffusion experiment with G-block (0.5 mg/ml) in section 3.1.2. In that experiment, tartrazine was added to Matrigel before G-block, whereas in the experiments with G-block (0.05 mg/ml and 5 mg/ml) the addition order was changed. The first significant difference in tartrazine diffusion in the diffusion experiment with G-block (0.5 mg/ml) was observed after 240 minutes, whereas in the two experiments with G-block concentrations of 0.05 mg/ml and 5 mg/ml, a significant difference in diffusion rate was evident after 90 minutes. In addition, the percentage diffused tartrazine in the experiment with G-block (0.5 mg/ml) was lower than both experiments with G-block (0.05 mg/ml and 5 mg/ml). The late onset of difference in tartrazine diffusion and the low effect of G-block (0.5 mg/ml) can be explained by the order of addition of tartrazine and G-block to Matrigel. By adding G-block before tartrazine, G-block is allowed to interact with Matrigel first. If there is a competitive binding of G-block and tartrazine to Matrigel, the addition of G-block before tartrazine would contribute to less bound tartrazine and a higher fraction of free tartrazine. Based on this hypothesis, one can question whether 0.5 mg/ml would have had a higher effect with earlier onset if the order was changed to G-block being added before tartrazine. Additional diffusion experiments with the change of order of addition would have to be performed in order to investigate this issue further.

From the results presented in Figure 3-4, it seems like the effect of G-block on tartrazine diffusion is dependent on the G-block concentration. Both experiments with G-block had significantly higher diffusion rate than the control and the highest G-block concentration of 5 mg/ml demonstrated a significant higher diffusion rate than the concentration of 0.05 mg/ml. In addition to demonstrate the largest effect on tartrazine diffusion rate, G-block (5 mg/ml) also had the highest observed diffused tartrazine percentage after 24 hours among the G-block test concentrations with 98 %. The concentration dependency can be explained by competitive binding of G-block and tartrazine, as higher G-block concentrations would result in a larger fraction of free tartrazine that will diffuse more rapidly than the bound fraction. Although the magnitude of difference in tartrazine diffusion at each sampling point seems to be G-block concentration dependent, the time of onset of difference is not. Both experiments with G-block concentration of 0.05 mg/ml and 5 mg/ml demonstrated the first significant difference of tartrazine diffusion after 90 minutes.

3.1.4 Verification of tartrazine diffusion from Matrigel with G-block

As mentioned in section 3.1.3, the tartrazine diffusion rate in Matrigel with G-block (5 mg/ml) was significantly higher than both the experiment with G-block (0.05 mg/ml) and the control. To validate the effect of G-block (5 mg/ml) on tartrazine diffusion from Matrigel, a similar diffusion experiment to the one described in section 3.1.3 was performed. The experiment was conducted according to section 2.2.1.4 Final protocol with Matrigel containing tartrazine (1 mM) and G-block (5 mg/ml, DP 12). 4 replicates were made and samples were removed every 30^{th} minute for 4.5 hours and after 24 hours. The concentration of tartrazine in each sample was determined by absorbance measurements using the standard curve presented in Figure B.2.1, Appendix B.2. The resulting tartrazine diffusion curve is presented in Figure 3-5, in addition to the diffusion curve of the original experiment and the control experiment described in section 3.1.1 Tartrazine diffusion curve. Raw data is given in Appendix C.2. Percentage diffused tartrazine at each sampling point was calculated based on the maximum potential concentration of diffused tartrazine in the saline solution. A concentration of 1 mM tartrazine in the gel corresponded to a maximum potential diffused tartrazine concentration of 0.133 mM in the saline solution. The percentage diffused tartrazine after 270 minutes and ≈ 24 hours is presented in Table 3-6.

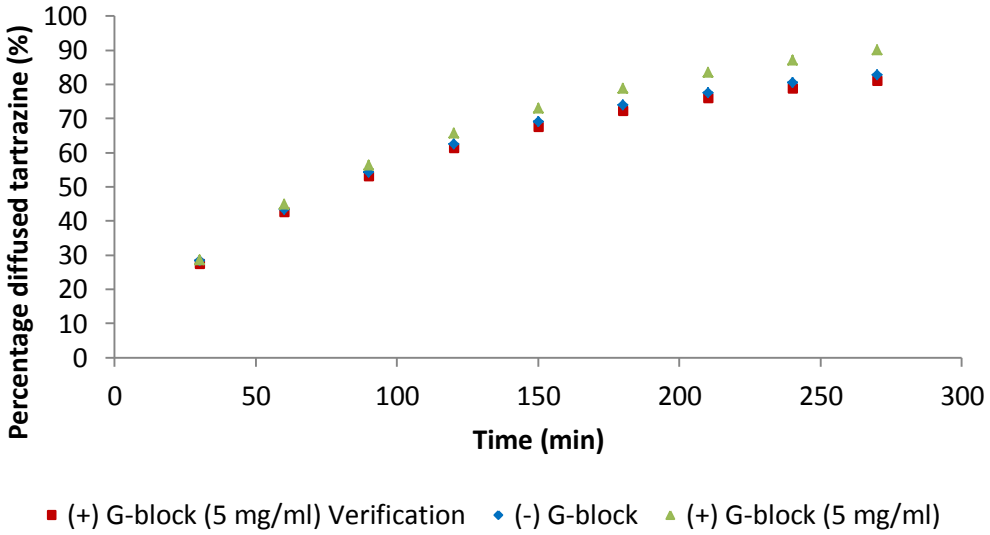

Figure 3-5. Tartrazine diffusion curve for control (without G-block) and diffusion experiments with tartrazine (1 mM) and; (1) G-block (0.05 mg/ml. DP 12) and (2) G-block (5 mg/ml. DP 12). Each point is given as the mean percentage diffused tartrazine of the replicates. Standard deviations of all curves were within the range from ±0.4 % to ±2.8 %.

Table 3-6. Percentage diffused tartrazine of maximum (0.133 mM in saline solution) after 270 minutes and ≈ 24 hours. Results are presented as mean percentage ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	82.9 ± 1.2	91.2 ± 1.2
(+) G-block (5 mg/ml) Verification	81.2 ± 2.3	89.1 ± 2.8
(+) G-block (5 mg/ml)	90.1 ± 0.8	98.0 ± 0.4

The tartrazine diffusion curve of the verification experiment was not significant different from the control at any sampling points, including the 24 hour sample, as demonstrated in Figure 3-5 and Table 3-6. The diffusion curve for the original experiment is also included to show the expected curve of the verification experiment. The failure of verification could be explained by the usage of a G-block solution stored for two months. Traces of growth, possibly bacterial or fungal, was found by investigating the G-block solution. It was assumed that these findings together with the long storage time could have affected the results, although it is uncertain how. The experiment was not repeated due to time limitations. However, the G-block concentration of 5 mg/ml was used in the following diffusion experiments due to the highest observed effect on tartrazine diffusion among the tested concentrations. New stock solutions of G-block were prepared for the following diffusion experiments.

3.1.5 Decreasing tartrazine concentration

Diffusion experiments with a reduction in tartrazine concentration and a fixed G-block concentration were performed to investigate if the effect of G-block on tartrazine diffusion from Matrigel was more evident. The G-block concentration of 5 mg/ml was used due to the largest observed effect on tartrazine diffusion compared to other test concentrations (see section 3.1.3). These experiments could also provide information about the interactions between tartrazine and Matrigel. There are at least two possible scenarios regarding the interactions between Matrigel and tartrazine. One hypothesis is that Matrigel may have a maximum binding capacity for tartrazine, resulting in the same absolute amount of tartrazine bound to Matrigel regardless of a change in tartrazine concentration in the gel. The second scenario is that it exists an equilibrium between free and bound tartrazine. Theoretically, the percentage bound tartrazine would in this case remain the same as the tartrazine concentration is altered. The theories were investigated by decreasing the tartrazine concentration of 1 mM utilized in previous diffusion experiments with 50 % and 75 % to 0.5 mM and 0.25 mM, respectively, in two additional diffusion experiments with respective control experiments.

A diffusion experiment with G-block (5 mg/ml, DP 12) and tartrazine (0.5 mM) in Matrigel, followed by a control experiment with tartrazine (0.5 mM) in Matrigel were conducted according to section 2.2.1.4 Final protocol with 3 replicates. In order to maintain a similar concentration gradient to previous performed experiments with a tartrazine concentration of 1 mM and obtain measurable tartrazine concentrations in each sample, it was necessary to reduce the sampling frequency. The sampling frequency was therefore reduced from nine to five sampling points (after 30, 90, 150, 210 and 270 minutes), in addition to a 24 hour sample, in both experiments.

The tartrazine concentration in Matrigel was further reduced to 0.25 mM in a diffusion experiment with G-block (5 mg/ml, DP 12) performed according to section 2.2.1.4 Final protocol with 3 replicates. A control experiment with only tartrazine (0.25 mM) in Matrigel was also conducted in a similar manner. Due to reduction of the tartrazine concentration compared to previous conducted diffusion experiments, the sampling frequency was reduced to three sampling points (after 60, 150 and 270 minutes) in an attempt to maintain the same tartrazine concentration gradient between Matrigel and the saline solution in the well as in

previous experiments. This was important for achieving measurable tartrazine concentration in each sample. Sampling after 24 hours was also conducted in both experiments.

The concentration of tartrazine in each sample was determined by absorbance measurements and the standard curve presented in Figure B.2.1, Appendix B.2. The resulting tartrazine diffusion curve from the diffusion experiment with 0.5 mM tartrazine is displayed in Figure 3-6, in addition to control data. The resulting tartrazine diffusion curves from the experiment with 0.25 mM tartrazine and the control experiment are presented in Figure 3-7. Raw data can be found in Appendix C.2. Percentage diffused tartrazine at each sampling point was calculated based on the maximum concentration of diffused tartrazine in the saline solution. A concentration of 0.5 mM tartrazine in the gel corresponded to a maximum potential diffused tartrazine concentration of 0.067 mM in the saline solution, whereas a concentration of 0.25 mM tartrazine in the gel corresponded to a maximum potential diffused tartrazine concentration of 0.033 mM in the saline solution. The percentage diffused tartrazine after 270 minutes and ≈ 24 hours in the experiments with 0.5 mM and 0.25 mM tartrazine is presented in Table 3-7 and Table 3-8, respectively.

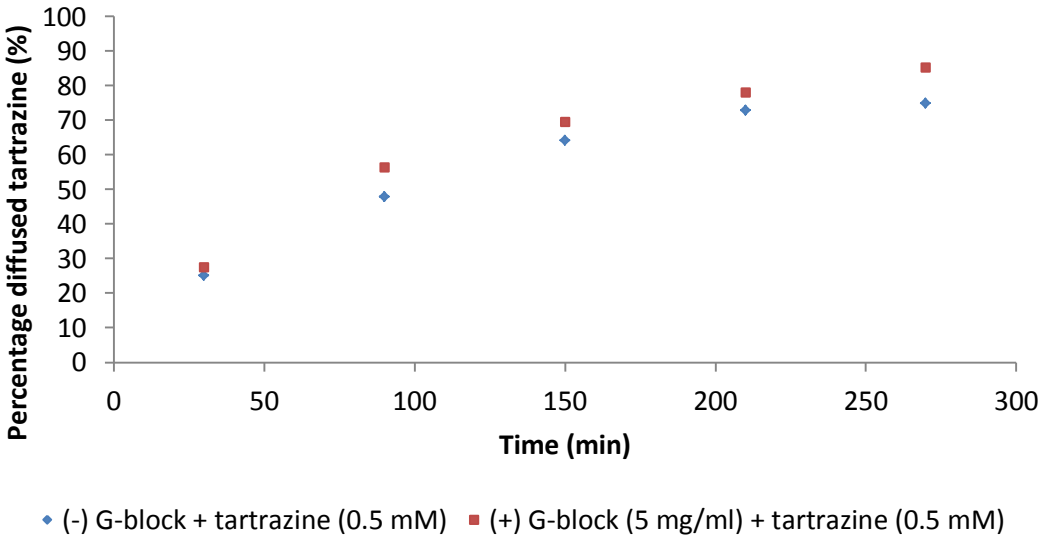

Figure 3-6. Tartrazine diffusion curve for control (without G-block) and diffusion experiment with G-block (5 mg/ml, DP 12). Both experiments utilized Matrigel with tartrazine (0.5 mM). Each point is given as the mean percentage diffused tartrazine of the replicates. Typical standard deviations for both experiments were in the range from ±0.9 % to ±4.2 %.

Table 3-7. Percentage diffused tartrazine of maximum (0.067 mM in saline solution) after 270 minutes and ≈ 24 hours in diffusion experiment with 0.5 mM tartrazine in Matrigel. Results are presented as mean percentage ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	75.0 ± 3.2	87.4 ± 1.0
(+) G-block (5 mg/ml)	85.2 ± 3.5	96.9 ± 4.2

The tartrazine diffusion results presented in Figure 3-6 and Table 3-7 demonstrate no significant difference in tartrazine diffusion from Matrigel between the experiment with G-block (5 mg/ml) and the control experiment at any sampling point (CI = 95 %, $p>0.05$), except for the sampling point after 270 minutes.

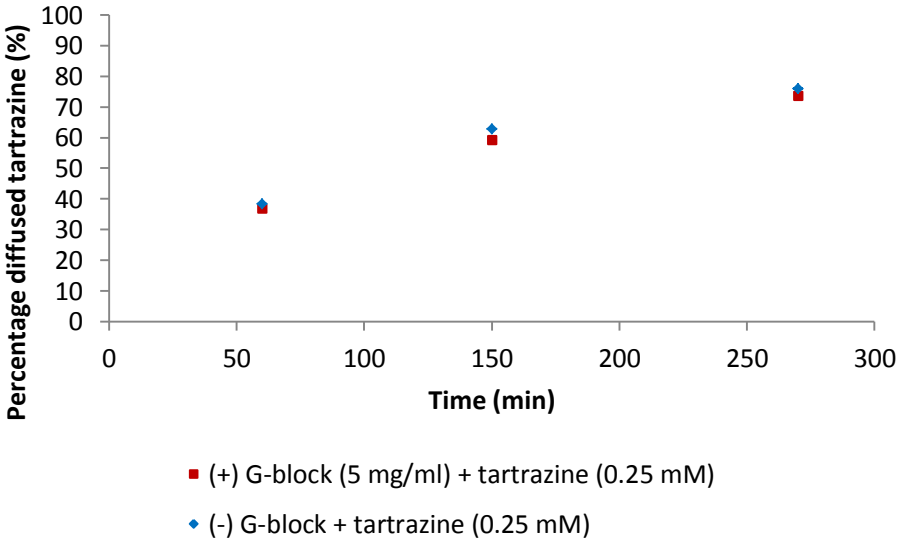

Figure 3-7. Tartrazine diffusion curve for control (without G-block) and diffusion experiments with G-block (5 mg/ml, DP 12). In both experiments Matrigel with tartrazine (0.25 mM) was used. Each point is given as the mean percentage diffused tartrazine of the replicates. Standard deviations for both experiments were in the range from ±2.2 % to ±5.6 %.

Table 3-8. Percentage diffused tartrazine of maximum (0.033 mM in saline solution) after 270 minutes and ≈ 24 hours in diffusion experiment with 0.25 mM tartrazine in Matrigel. Results are presented as mean percentage ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	76.1 ± 4.1	105.3 ± 5.0
(+) G-block (5 mg/ml)	73.7 ± 5.6	85.0 ± 5.6

The tartrazine diffusion curves in Figure 3-7 demonstrate no significant difference in diffusion rate between the experiment with G-block (5 mg/ml) and the control experiment at any sampling points (CI = 95 %, $p>0.05$). However, the tartrazine diffusion rate of the control is significant higher than the G-block experiment at the 24 hour sampling point (CI = 95 %, $p<0.05$) (Table 3-8).

A summary of the percentage diffused tartrazine in the controls and experiments with G-block (5 mg/ml) with the tartrazine concentrations 1 mM, 0.5 mM and 0.25 mM is presented in Table 3-9.

Table 3-9. Percentage diffused tartrazine after 270 minutes and 24 hours for controls and diffusions experiments with Matrigel containing G-block (5 mg/ml) and tartrazine concentrations of 1 mM, 0.5 mM and 0.25 mM. Results are presented as mean percentage ± standard deviation.

	270 min		24 hours	
Tartrazine concentration (mM)	G-block (5 mg/ml)	Control	G-block (5 mg/ml)	Control
1	90.1 ± 0.8	82.9 ± 1.2	98.0 ± 0.4	91.2 ± 1.2
0.5	85.2 ± 3.5	75.0 ± 3.2	96.9 ± 4.2	87.4 ± 1.0
0.25	73.7 ± 5.6	76.1 ± 4.1	85.0 ± 5.6	105.3 ± 5.0

The attempt to maintain a similar concentration gradient as diffusion experiments with tartrazine concentration of 1 mM was successful in both experiments with 0.5 mM and 0.25 mM tartrazine, as measureable tartrazine concentrations were obtained at each sampling point.

A result that has to be questioned in the two diffusion experiments with reduced tartrazine concentration is the observed percentage diffused tartrazine exceeding 100 %. This is observed in the control of the 0.25 mM tartrazine experiment and in the G-block diffusion experiment with 0.5 mM tartrazine, both at the 24 hour sampling point. When comparing the control of 0.25 mM tartrazine to the controls of 0.5 mM and 1 mM tartrazine (Table 3-9), there are no other controls with tartrazine diffusion exceeding 100 %. Two possible scenarios can explain the observed tartrazine diffusion above 100 %. One explanation might be an experimental error resulting in additional added tartrazine, either from the start of the experiment or during sampling. To investigate this further, the experiment should have been

repeated. A second explanation considers the fact that Matrigel contain phenol red, which might affect the absorbance measurements of tartrazine. Phenol red has a maximum absorbance of 430 – 435 nm at pH 6.5 (Chemie, 2013), which is close to the determined maximum absorbance of tartrazine at 426 nm. However, even if phenol red affects the absorbance measurements, the effect would be expected to be similar for all diffusion experiments as the volume of Matrigel was similar in all experiments. To study the possible background absorbance of phenol red, control diffusion experiments with Matrigel should be performed.

The network of Matrigel, tartrazine and G-block is complex with many possible interactions. As mentioned earlier, two possible interaction scenarios between Matrigel and tartrazine were hypothesized. One theory is that the Matrigel has a maximum binding capacity of tartrazine, whereas the second theory involves an equilibrium between free and bound tartrazine in Matrigel. Binding of tartrazine in the gel network of Matrigel is illustrated in Figure 3-8.

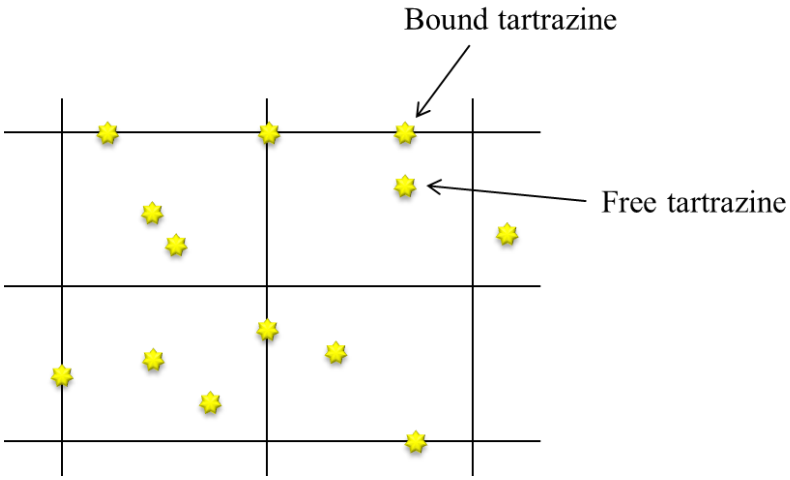

Figure 3-8. Illustration of the hypothesis of bound and free tartrazine in the gel network of Matrigel.

The theories were investigated by decreasing the tartrazine concentration of 1 mM utilized in previous diffusion experiments with 50 % and 75 %, while keeping the G-block concentration of 5 mg/ml constant. An overview of the expected results for the two theories, in addition to the observed results is presented in Table 3-10. The observed results for the diffusion experiment with 0.25 mM is not presented as the reliability of the data can be questioned due to diffusion values exceeding 100%, as discussed previously. In addition, the results demonstrated no effect of G-block as the control had higher tartrazine diffusion rates.

Table 3-10. Expected results for the two hypothesized theories: 1) a specific binding capacity of tartrazine in Matrigel and 2) equilibrium between bound and free tartrazine. The observed results are based on measured tartrazine diffusion after 24 hours in two diffusion experiments with 1 mM and 0.5 mM tartrazine, respectively.

	Bound (%)	Bound, released by G-block (%)	Free (%)
Observed with 1 mM tartrazine	**2**	**7**	**91**
Expected with 0.5 mM tartrazine, equilibrium	>2	7	91
Expected with 0.5 mM tartrazine, binding capacity	>2	14	84
Observed with 0.5 mM tartrazine	**3**	**10**	**87**
Expected with 0.25 mM tartrazine, equilibrium	>2	7	91
Expected with 0.25 mM tartrazine, binding capacity	>2	28	70

The control experiment with 1 mM tartrazine demonstrated a bound tartrazine fraction of 9 % after 24 hours. The bound fraction was reduced to 2 % in the diffusion experiment with G-block (5 mg/ml), resulting in dissolution of 7 % of the bound tartrazine. The last fraction of tartrazine of 91 % is considered not to be affected by G-block and is referred to as free. If Matrigel has a certain binding capacity for tartrazine, the percentage tartrazine released by G-block is expected to double from 7 % to 14 % when the tartrazine concentration of 1 mM is reduced by 50 % to 0.5 mM. Furthermore, a reduction of tartrazine concentration from 0.5 to 0.25 mM is expected to cause a doubling from 14 % to 28 % released tartrazine by G-block according to the theory of an existing equilibrium. On the other hand, if there exists an equilibrium between bound and free tartrazine, the percentage of tartrazine released by G-block is expected to remain the same as the tartrazine concentration is reduced.

The percentage bound tartrazine in Matrigel that is not affected by G-block is expected to increase when the tartrazine concentration is reduced. This will be expected for both theories, although a higher increase may occur if the Matrigel has a certain binding capacity for tartrazine. Because this value is difficult to predict, the expected value was set to be higher than 2 %, which was the percentage bound tartrazine not affected by G-block in the experiment with 1 mM tartrazine.

The results of the diffusion experiment with tartrazine (0.5 mM) and G-block (5 mg/ml) showed that the percentage bound tartrazine after 24 hours was 3 %, whereas 10 % of the bound tartrazine in the control was released by G-block. The values are in between the expected values for both theories postulated, suggesting that the interactions of Matrigel and tartrazine can be explained by a mix of the binding capacity and equilibrium theory. The bound fraction of tartrazine was slightly increased from 2 % to 3 %. The results illustrate that Matrigel is a complex biological system with interactions that has to be explained by more theories than those postulated in this thesis.

3.1.6 Control fluorescence diffusion experiments

Due to the use of fluorescent labelled Alexa Fluor® 488 IgG as the second model compound in diffusion experiments, two control diffusion experiments were conducted in order to investigate the amount of background fluorescence. In the first control experiment, saline replaced the volume of model compound and G-block in Matrigel. The purpose of this experiment was to investigate whether the components of Matrigel contributed to any background fluorescence. In the second control experiment, Matrigel with G-block (5 mg/ml) was utilized to examine if G-block increased the release of potential fluorescent components from Matrigel. Both experiments were carried out according to section 2.2.1.4 Final protocol with 5 replicates. Since there was no model compound involved in these experiments, the sampling frequency was reduced to four sampling points (after 60, 120, 180 and 240 minutes) in a period of 4 hours, in addition to a 24 hour sample. The measured fluorescence values in both control experiments are presented in Table 3-11.

Table 3-11. Measured fluorescence values by fluorescence spectroscopy for two control experiments; one with Matrigel and saline, the other with Matrigel and G-block (5 mg/ml). The results are presented as the mean fluorescence of the five replicates ± the standard deviation.

Time (min)	Fluorescence	Fluorescence
	Matrigel, saline	Matrigel, G-block (5 mg/ml)
60	0.135 ± 0.009	0.141 ± 0.007
120	0.138 ± 0.007	0.139 ± 0.003
180	0.124 ± 0.009	0.129 ± 0.006
240	0.116 ± 0.007	0.131 ± 0.008
1440	0.130 ± 0.011	0.126 ± 0.008

The results presented in Table 3-11 demonstrate no background fluorescence of importance from components of the Matrigel, as the lowest measured fluorescence value in samples from IgG diffusion experiments was 3.810 (section 3.1.7 and 3.1.8). The measured background fluorescence values are similar to the measured fluorescence of a saline sample in the IgG diffusion experiments, which was around 0.120. Since the background fluorescence values were low compared to the experimental measurements and close to equal at each point of sampling, they were not corrected for in the diffusion experiments with IgG. Due to the low

control fluorescence values, it can be questioned whether there is background fluorescence or if the measured fluorescence reflects the limits of the fluorometer, as some of the incident light may reach the detector. There is no difference in fluorescence between the two control experiments. This means that if the measured fluorescence was a result of fluorescent compounds in Matrigel, G-block is not increasing the release of these components.

3.1.7 The effect of G-block on diffusion of Alexa Fluor® 488 IgG from Matrigel

To study the effect of G-block on diffusion of macromolecules from Matrigel, a diffusion experiment with Matrigel containing the model compound Alexa Fluor® 488 IgG (0.25 mg/ml) and G-block (5 mg/ml, DP 12) was performed. A diffusion experiment with only Alexa Fluor® 488 IgG (0.25 mg/ml) in Matrigel served as the control experiment. Both experiments were conducted according to section 2.2.1.4 Final protocol with 5 replicates. Similar to most of the diffusion experiments performed with tartrazine as model compound, G-block (5 mg/ml) was added to Matrigel prior to Alexa Fluor® 488 IgG. Sampling was carried out every 30th minute for 4.5 hours, in addition to a 24 hour sample. The concentration of IgG in each sample was determined by fluorescence spectroscopy and the standard curve provided in Figure B.3.1, Appendix B.3. The Alexa Fluor® 488 IgG diffusion curve for the experiment with G-block (5 mg/ml) and the control experiment is presented in Figure 3-9. Raw data can be found in Appendix C.2. The percentage diffused Alexa Fluor® 488 IgG at each sampling point was calculated based on the maximum potential concentration of diffused IgG in the saline solution. An IgG concentration of 0.25 mg/ml in the gel corresponded to a maximum potential diffused IgG concentration of 33.3 µg/ml in the saline solution. Calculation example can be found in Appendix C.1. The percentage diffused Alexa Fluor® 488 IgG after 270 minutes and ≈ 24 hours is presented in Table 3-12.

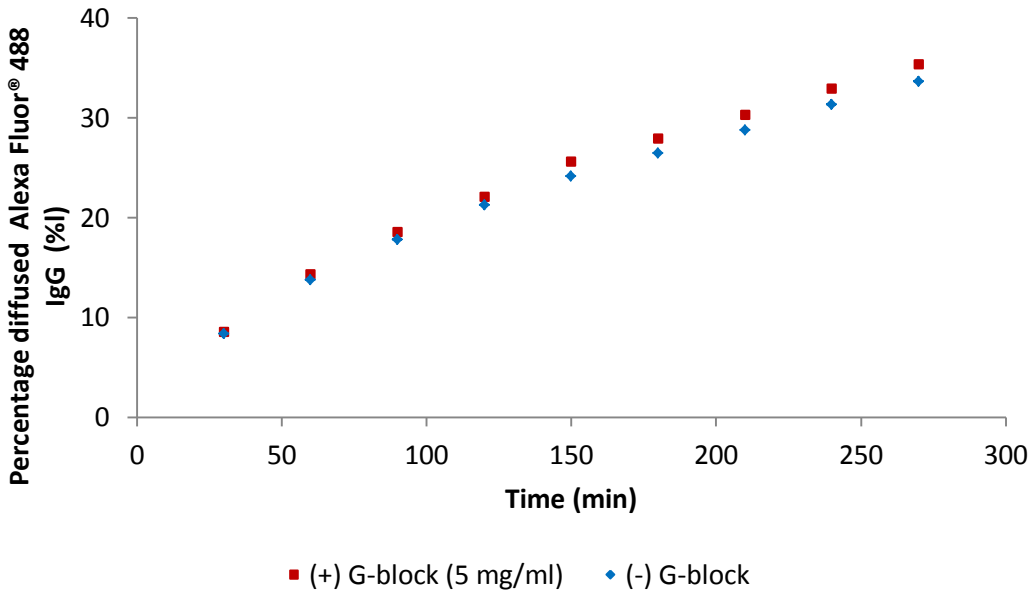

Figure 3-9. Diffusion curve of Alexa Fluor® 488 IgG (0.25 mg/ml) in Matrigel for control diffusion experiment (without G-block) and diffusion experiment with G-block (5 mg/ml. DP 12). Each point is given as the mean percentage diffused IgG of the 5 replicates. Standard deviations of both experiments were in the range from ±0.1 % to ±3.3 %.

Table 3-12. Percentage diffused Alexa Fluor® 488 IgG of maximum (33.3 µg/ml in saline solution) after 270 minutes and ≈ 24 hours. Results are presented as mean percentage ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	33.7 ± 0.3	85.4 ± 1.2
(+) G-block (5 mg/ml)	35.4 ± 1.0	92.2 ± 3.3

As demonstrated in Figure 3-9, Alexa Fluor® 488 IgG diffuses down its concentration gradient from Matrigel into the saline solution during the diffusion experiment. The diffusion of IgG in the first 4.5 hours follows an approximate linear trend. There is no significant difference between the diffusion rates of the experiment with G-block (5 mg/ml) and the control experiment at the two first sample points (after 30 and 60 minutes) (CI = 95 %, $p>0.05$). However, the experiment with G-block (5 mg/ml) demonstrates a significantly higher diffusion rate than the control from 90 minutes and up to the 24 hour sample (CI = 95 %, $p<0.05$). Results from the statistical analyses are summarized in Table 3-13. Further details can be found in Appendix D.

Table 3-13. Statistical analyses of Alexa Fluor® 488 IgG diffusion in diffusion experiment with G-block concentration 5 mg/ml compared to control. The concentration of IgG in Matrigel was 0.25 mg/ml. Results are presented as p-values with significant difference labelled with *. CI = 95 %.

Sampling time (min)	p-value
30	0.567
60	0.056
90	0.038*
120	0.037*
150	0.002*
180	0.003*
210	0.010*
240	0.015*
270	0.013*
≈ 1440 (24 hours)	0.007*

Alexa Fluor 488® immunoglobulin G (IgG) was used as a model compound in this thesis to investigate the diffusion of macromolecules from Matrigel. IgG molecules are a class of immunoglobulins with a molecular weight of 150 000 Da and are produced as part of the secondary immune response to an antigen (Life Technologies(B)). By using fluorescent labelled IgG the concentration could easily be determined by fluorescence spectroscopy, which is a method providing high sensitivity detection.

The Matrigel network is complex and may interact with IgG in several possible ways. As discussed previously with tartrazine as model compound, it is possible that a fraction of IgG is bound to Matrigel, whereas the other part is free and diffuses more rapidly into the saline solution. Based on the results (Table 3-13), it is suggested that mainly the free fraction of IgG in Matrigel diffuses in the course of the first 60 minutes, as there is no significant difference between the experiment with G-block (5 mg/ml) and the control in this time interval. A significant higher diffusion rate of Alexa Fluor 488® IgG in the experiment with G-block compared to the control was observed from 90 minutes and up to 24 hours. Several interactions may be the cause of this effect, suggesting competitive binding of G-block to Matrigel as one of them. If G-block binds Matrigel instead of IgG, the fraction of free IgG will increase.

Another theory is that G-block may affect the Matrigel network structure in a way that reduces the steric hindrance of IgG, thus enabling an increased diffusion rate. Steric hindrance is expected to be of larger importance with IgG as model compound than tartrazine, as IgG has a larger size than tartrazine. A larger proportion of IgG molecules is therefore expected to be sterically trapped within the Matrigel. Compared to the diffusion experiments with tartrazine, the diffusion rate of Alexa Fluor 488® IgG from Matrigel was lower than tartrazine. The diffusion of IgG in the control experiment was 34 % after 270 minutes (Table 3-12), whereas percentage diffused tartrazine in the control with 1 mM tartrazine was 83 % after 270 minutes (see section 3.1.1). The difference in diffusion rate was expected as IgG has a larger size and is more hydrophobic than tartrazine. However, the difference is reduced to 85 % diffused IgG and 91 % diffused tartrazine after 24 hours. Although it is impossible to state anything about the diffusion of tartrazine and IgG in the time interval from 270 minutes to 24 hour, the results suggests that although the diffusion rate of IgG is lower than tartrazine, the IgG diffusion rate is more stable throughout the 24 hours. Tartrazine is a hydrophilic, small molecule that is diffusing rapidly from Matrigel in the first 4.5 hours of the experiment. It is assumed that the diffusion curve of IgG will level off at some point during the experiment as the concentration gradient is reduced.

3.1.8 Decreasing Alexa Fluor® 488 IgG concentration

A diffusion experiment with a reduced Alexa Fluor® 488 IgG concentration (0.05 mg/ml) and G-block (5 mg/ml, DP 12) was performed to investigate if the effect of G-block on IgG diffusion from Matrigel was more evident. The experiment and a control experiment with Matrigel containing only Alexa Fluor® 488 IgG (0.05 mg/ml) were carried out according to section 2.2.1.4 Final protocol. 5 replicates were made in each experiment and the sampling frequency was every 30th minute for 4.5 hours, in addition to a 24 hour sample. Fluorescence spectroscopy was utilized to determine the concentration of IgG in each sample, in addition to the standard curve provided in Figure B.3.1, Appendix B.3. The frequency of sampling was not reduced as was done in the experiments with reduced tartrazine concentration because the IgG sample concentrations were assumed to have fluorescence values within the linear region of the standard curve. This was confirmed in the experiment. The resulting diffusion curves of Alexa Fluor® 488 IgG from both experiments are presented in Figure 3-10. Raw data can be found in Appendix C.2. The percentage diffused Alexa Fluor® 488 IgG at each sampling point was calculated based on the maximum potential concentration of diffused IgG in the saline solution. An IgG concentration of 0.05 mg/ml in the gel corresponded to a maximum potential diffused IgG concentration of 6.66 μg/ml in the saline solution. The percentage diffused Alexa Fluor® 488 IgG after 270 minutes and ≈ 24 hours is presented in Table 3-14.

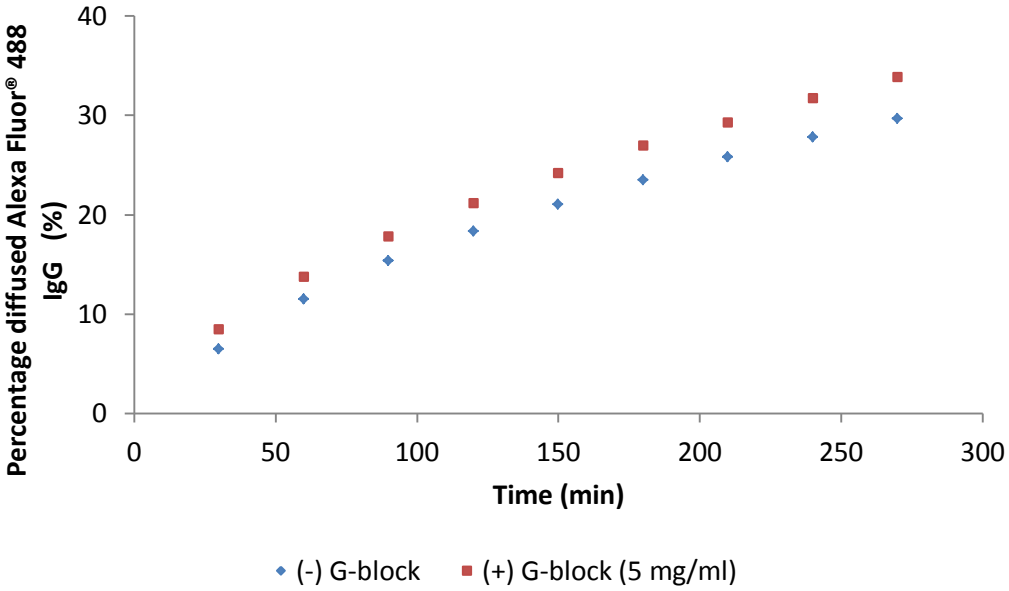

Figure 3-10. Diffusion curve of Alexa Fluor® 488 IgG (0.05 mg/ml in gel) in Matrigel for control diffusion experiment (without G-block) and diffusion experiment with G-block (5 mg/ml, DP 12). Each point is given as the mean percentage diffused IgG of the 5 replicates. Standard deviations of both experiments were in the range from ±0.2 % to ±5 %.

Table 3-14. Percentage diffused Alexa Fluor® 488 IgG of maximum (6.66 µg/ml in saline solution) after 270 minutes and ≈ 24 hours in control and G-block diffusion experiment. Results are presented as mean value ± standard deviation.

	270 min (%)	≈ 24 hours (%)
Control	29.7 ± 0.5	77.0 ± 2.1
(+) G-block (5 mg/ml)	33.9 ± 1.5	88.2 ± 5.0

Figure 3-10 and Table 3-14 demonstrate a significant higher diffusion rate of Alexa Fluor® 488 IgG (0.05 mg/ml) in the diffusion experiment with G-block (5 mg/ml) compared to the control experiment at all sampling points, including the 24 hour sample (CI = 95 %, $p<0.05$).

A summary of the percentage diffused IgG in controls and experiments with G-block (5 mg/ml) with the IgG concentration of 0.25 mg/ml and 0.05 mg/m is presented in Table 3-15.

Table 3-15. Percentage diffused IgG after 270 minutes and 24 hours for controls and diffusions experiments with Matrigel containing G-block (5 mg/ml) and Alexa Fluor® 488 IgG concentrations of 0.25 mg/ml and 0.05 mg/ml. Results are presented as mean percentage ± standard deviation.

	270 min		24 hours	
IgG concentration (mg/ml)	**G-block (5 mg/ml)**	**Control**	**G-block (5 mg/ml)**	**Control**
0.25	35.4 ± 1.0	33.7 ± 0.3	92.2 ± 3.3	85.4 ± 1.2
0.05	33.9 ± 1.5	29.7 ± 0.5	88.2 ± 5.0	77.0 ± 2.1

By studying how G-block affects the diffusion of IgG (Table 3-15), it can be investigated whether Matrigel has a certain binding capacity of IgG or if it exists an equilibrium between free and bound IgG. The two theories were investigated by performing diffusion experiments with the IgG concentration of 0.25 mg/ml and to 0.05 mg/ml, both with a constant G-block concentration of 5 mg/ml. An overview of the expected results for the two theories, in addition to the observed results is presented in Table 3-16.

Table 3-16. Expected results for the two hypothesized theories: 1) a specific binding capacity of IgG in Matrigel and 2) equilibrium between bound and free IgG. The observed results are based on measured IgG diffusion after 24 hours in two diffusion experiments with the IgG concentration of 0.25 mg/ml and 0.05 mg/ml, respectively.

	Bound (%)	Bound, released by G-block (%)	Free (%)
Observed with 0.25 mg/ml IgG	**8**	**7**	**85**
Expected with 0.05 mg/ml IgG, binding capacity	>8	35	57
Expected with 0.05 mg/ml IgG, equilibrium	>8	7	85
Observed with 0.05 mg/ml IgG	**12**	**11**	**77**

The control experiment with 0.25 mg/ml IgG demonstrated a bound IgG fraction of 15 % in Matrigel after 24 hours. The bound fraction was reduced to 8 % in the diffusion experiment with G-block, resulting in dissolution of 7 % of bound IgG. The last fraction of IgG of 85 % is considered not to be affected by G-block and is referred to as free. If it exists an equilibrium between bound and free IgG in Matrigel, the percentage of IgG released by G-block is expected to remain the same as the IgG concentration is reduced. However, if Matrigel has a certain binding capacity of IgG, the percentage IgG released by G-block is expected to increase from 7 % to 35 % when the IgG concentration of 0.25 mg/ml is reduced to 0.05 mg/ml.

The percentage bound IgG in Matrigel that is not affected by G-block is expected to increase when the IgG concentration is reduced. This will be expected for both theories, although a higher increase may occur if the Matrigel has a certain binding capacity for IgG. Because this value is difficult to predict, the expected value was set to be higher than 8 %, which was the percentage bound IgG not affected by G-block in the experiment with 0.25 mg/ml IgG.

The results of the diffusion experiment with IgG (0.05 mg/ml) and G-block (5 mg/ml) showed that the percentage bound IgG after 24 hours was 12 %, whereas 11 % of the bound IgG in the control was released by G-block. The values are close to the expected values for the theory of equilibrium, suggesting an equilibrium between bound and free IgG in Matrigel. It was also observed an increase of bound IgG from 8 % to 12 %. However, due to the complexity of this biological system, this single theory is most likely not sufficient to explain the interactions between IgG and Matrigel.

3.1.9 Summary diffusion experiments

A diffusion experiment was developed and optimized in order to study the effect of G-block on diffusion of molecules out of an extracellular matrix. The method utilized Matrigel as model for ECM, whereas the dye tartrazine and Alexa Fluor® 488 IgG served as model compounds in separate experiments. The model compounds were incorporated in Matrigel with or without G-block and allowed to diffuse through a polycarbonate filter into a saline solution. Samples were collected at various times from the saline solution to determine the concentration of diffused model compound.

The effect of three different G-block concentrations on the diffusion of tartrazine (1 mM) from Matrigel was tested; 0.05 mg/ml, 0.5 mg/ml and 5 mg/ml. The concentrations of 0.05 mg/ml and 5 mg/ml demonstrated a significant higher diffusion rate of tartrazine compared to the control experiment. In addition, 5 mg/ml showed a significant higher diffusion rate than 0.05 mg/ml, suggesting a concentration dependent effect of G-block on tartrazine diffusion. The concentration of 0.5 mg/ml did not demonstrate an effect of G-block, which might be caused by the different addition order of tartrazine and G-block compared to the other two experiments. In this experiment, tartrazine was added prior to G-block, possibly disturbing the interactions between G-block and Matrigel. The G-block concentration of 5 mg/ml was used in the following experiments due to the largest observed effect on tartrazine diffusion from Matrigel. Alexa Fluor® 488 IgG was utilized as a second model compound to study diffusion of macromolecules from Matrigel. G-block (5 mg/ml) demonstrated a significant higher diffusion rate of IgG (0.25 mg/ml) compared to the control.

The concentrations of tartrazine and IgG were reduced to investigate if the effect of G-block was more evident and to achieve a better understanding of the interactions between the model compounds and Matrigel. Two theories explaining the interactions between Matrigel and the model compounds were postulated. The first assumed a specific binding capacity of tartrazine and IgG in Matrigel, whereas the other considered the existence of an equilibrium between free and bound tartrazine and IgG in Matrigel. The concentration of tartrazine was reduced by 50 % and 75 % to 0.5 mM and 0.25 mM in two separate experiments, which both demonstrated no significant effect of G-block on tartrazine diffusion from Matrigel. However, the results indicated that the interactions between Matrigel and tartrazine can be explained by a mix of the two theories hypothesized. The concentration of IgG was reduced by 80 % to

0.05 mg/ml in a diffusion experiment which demonstrated a significant higher diffusion of IgG in Matrigel with G-block. In addition, the results indicated an equilibrium between free and bound IgG in Matrigel. The results reflected the fact that Matrigel is a complex biological system with interactions that has to be explained by more comprehensive theories than those postulated in this thesis.

In vitro methods for studying molecule mobility in biogels can be divided into two main groups; methods measuring the overall molecule transport and methods that measure the molecule mobility in local microregions of the biogels (Sanders *et al.*, 2000a). The diffusion experiment method developed in this thesis is based on a diffusion chamber method, which measures the overall transport of molecules from Matrigel. The method involves monitoring changes in concentration in donor and acceptor compartments as a function of time. To investigate local molecule mobility in Matrigel, fluorescence recovery after photobleaching (FRAP) could have been utilized. Diffusion assays with vertical diffusion chambers have been used in a number of studies to investigate transport of molecules across synthetic and biologic barriers. The diffusion chamber method is a common method to study molecular diffusion across mucus barriers (Khanvilkar *et al.*, 2001). In particular, nanoparticle diffusion through cystic fibrosis has been thoroughly investigated by utilizing diffusion assays (Broughton-Head *et al.*, 2007, Sanders *et al.*, 2000b)

A limited number of studies utilizing diffusion assays in the study of molecule transport in ECM gels has been reported. A study conducted by Galgoczy *et al.* has similarities to the developed diffusion experiment in this thesis. The study included development of a simple diffusion assay for studying diffusion of FITC-dextrans (4-70 kDa) in acellular ECM gels (Matrigel, fibrin and type I collagen) by utilizing Transwell and fluorescence intensity measurements (Galgoczy *et al.*, 2014). However, the dextrans were placed on top of the gels in contrast to being incorporated in the gels. Placing the compounds on top of the gel may potentially involve additional error sources as the method requires a uniform and intact gel surface to achieve comparable diffusion experiments.

3.2 Rheological characterization of Matrigel

3.2.1 Rheological measurements

Rheological characterization of various Matrigel samples was performed to investigate if G-block can affect the Matrigel matrix. Oscillatory measurements were run to determine the rheological profile of Matrigel with and without G-block. The composition of the different gel samples rheologically tested is presented in Table 3-17. Gel 4 and 5 had the same composition as gels utilized in the diffusion experiments. Further details of the preparation of gels utilized in the rheological measurements can be found in section 2.2.2 Rheological measurements. Example of rheological raw data can be found in Appendix E. Raw data for all experiments are enclosed in a zip-file.

Table 3-17. Overview of composition of gels used in rheological measurements.

Gel	Composition
1	Matrigel
2	Matrigel, saline
3	Matrigel, G-block (5 mg/ml in gel)
4	Matrigel, tartrazine (1 mM in gel)
5	Matrigel, G-block (5 mg/ml in gel), tartrazine (1 mM in gel)

3.2.2 Determining the rheological characterization method

In order to study and compare the rheological properties of the gels utilized in the diffusion experiments, a suitable gel setting method had to be selected. There were two possible options of methods appropriate for this purpose. The first option was to mimic the gel setting in the diffusion experiments by quickly increasing the temperature from 4 ºC to 37 ºC prior to oscillatory measurements. This method is from now on referred to as the temperature ramp. The second option was to perform oscillatory measurements with a temperature gradient from 4 ºC to 37 ºC. By gradually increasing the temperature the gel components are allowed to interact in a favorable fashion before the gel is set. This method allows the study of fundamental gel kinetics. However, this method would not resemble the gel setting in the diffusion experiments in the same way as for the temperature ramp.

3.2.2.1 Oscillation strain control

The two options were tested by conducting oscillatory measurements on Matrigel in two separate experiments. Matrigel was thawed according to the protocol (Appendix A) and directly added to the plate of the rheometer before starting the measurements. Both options were tested by running oscillation strain control measurements at a fixed frequency of 1 Hz and strain of $5*10^{-3}$, shown to be within the linear viscoelastic region (Figure 3-15 and Figure 3-16, strain sweeps). The setting curve of Matrigel based on the measurements from the temperature ramp is displayed in Figure 3-11. The setting curve of Matrigel with the temperature increased gradually from 4 ºC to 37 ºC over a period of 35 minutes is presented in Figure 3-12. The measurements of Matrigel from the temperature ramp were run for 21 minutes. while Matrigel from the temperature gradient was monitored for 293 minutes due to longer time before establishing equilibrium.

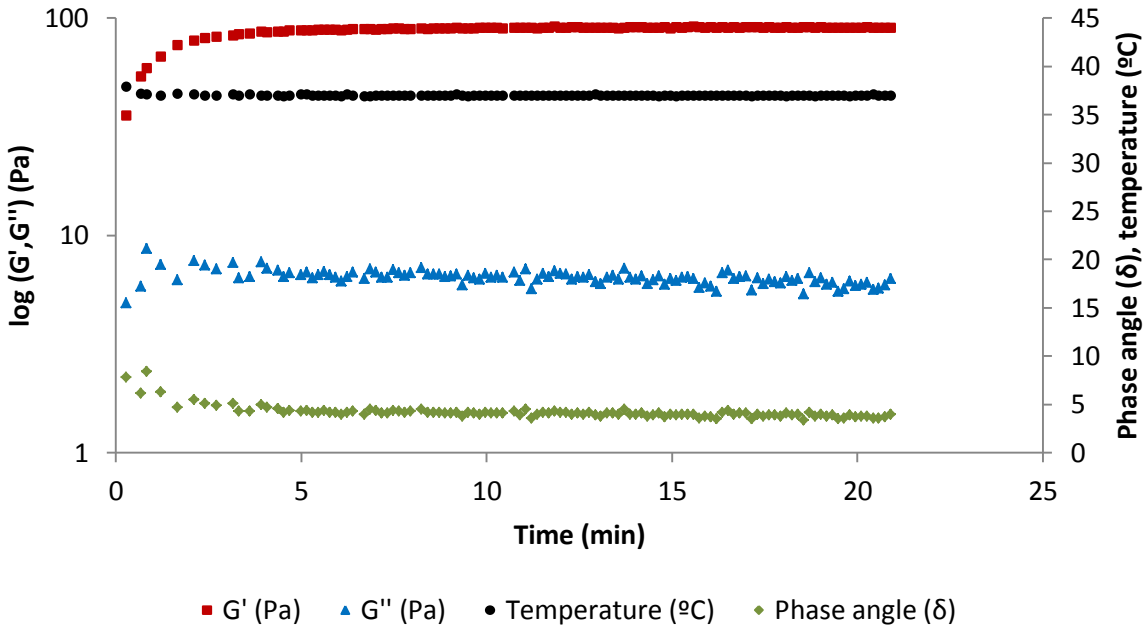

Figure 3-11. Setting curve of Matrigel from temperature ramp. Measurements were run at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 21 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements.

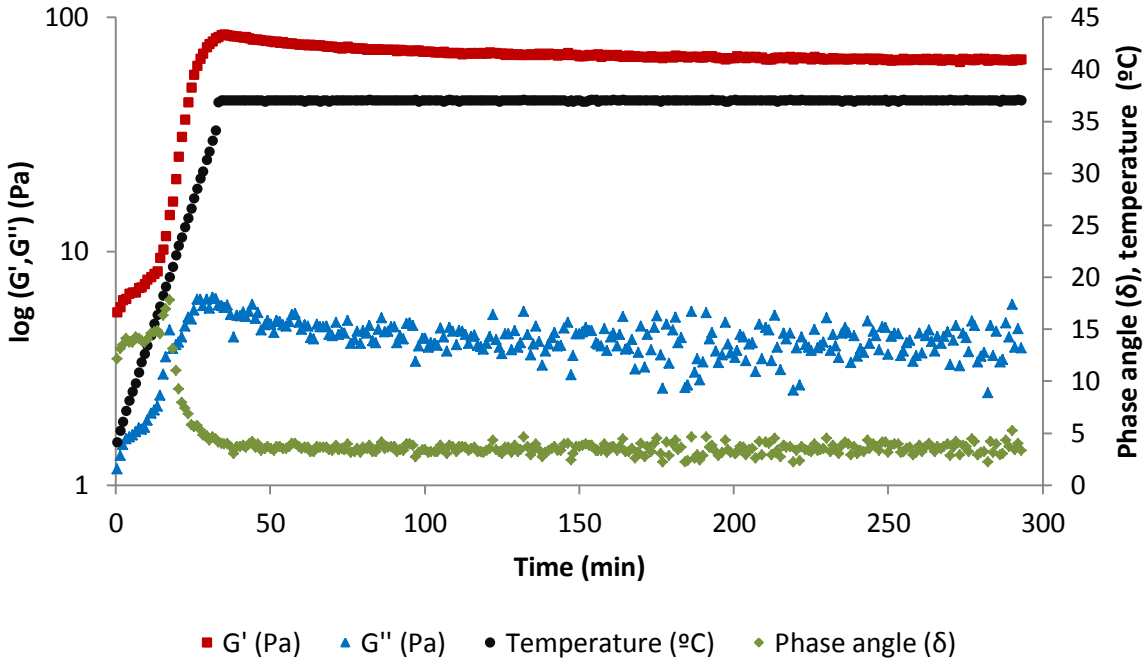

Figure 3-12. Setting curve of Matrigel from temperature gradient. Measurements were run at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 293 minutes. The temperature was increased from 4 ºC to 37 ºC with a gradient of 1 ºC/min during measurements.

Figure 3-11 demonstrates the setting curve of Matrigel from the temperature ramp, in which G' increases and levels off at 90 Pa as an equilibrium is established within the first 5 minutes of the experiment. In the setting curve of Matrigel from the temperature gradient (Figure 3-12), G' increases to a maximum value of 84 Pa before it decreases and stabilizes around 66 Pa after 250 minutes.

The storage modulus (G') was larger than the loss modulus (G'') throughout both experiments. The phase angles were below 20º, indicating a solid-like behaviour from the start of the measurements. However, the sample was observed as a liquid before application. A liquid is characterized by higher values of G'' than G', whereas a true gel system typically displays a parallel relationship between the moduli with G' > G''. As the liquid sample is set into a gel, the transition is observed by a cross-over between G' and G'' (Picout and Ross-Murphy, 2003). The reason why this transition was not observed is most likely due to the frequency dependent behaviour of the material. The measurements were performed at a frequency of 1 Hz, which is a higher frequency than the frequency in the visual observation of the sample. The liquid sample can therefore display solid-like behaviour during measurements. An additional explanation might be that Matrigel has a surface tension measured as a solid surface by the rheometer at small strains.

This setting curve of the temperature gradient Matrigel shows typical features of syneresis; the spontaneous contraction of a gel, followed by expulsion of liquid (Scherer, 1989). The process occurs without the application of external forces. Syneresis can occur even if evaporation is prevented by covering the gel in liquid, such as low viscosity silicone oil. Despite the great interest, the mechanism of syneresis is not fully understood (Ako, 2015, Scherer, 1989). As a result of syneresis, the instrument reading will be distorted due to lack of contact between the gel surface and the metal surfaces of the rheometer geometry. This is also referred to as "slip" and can explain the decrease in G' before reaching equilibrium in Figure 3-12 (Picout and Ross-Murphy, 2003).

The different setting curves for the two experiments can be explained by the different temperature gradients. In the temperature ramp, the temperature was rapidly ramped from 4 ºC to 37 ºC prior to measurements. This may cause the gel to set before the most favorable interactions between the components are allowed to occur. In the temperature gradient, the

temperature was gradually increased from 4 ºC to 37 ºC with a gradient of 1 ºC/min. The components of the gel solution were therefore allowed to interact in a favorable manner before the gel was set, leading to the production of an organized gel network exhibiting syneresis with water as the expulsed liquid.

Based on the two different setting curves as a result of the two different methods, the temperature ramp was determined to be the best option for further characterization of various Matrigel gels. The temperature ramp method resulted in a setting curve with a clear established equilibrium, enabling comparison of Matrigel gels. Another advantage was that the method provided similar gel setting as in the diffusion experiments. The syneresis observed in the setting of Matrigel from the temperature gradient does not produce a good basis for comparison as equilibrium is not established immediately. It is challenging to compare gels exhibiting syneresis because it is difficult to predict where in the process each gel is. The temperature gradient method was therefore disregarded as a suitable method for characterization of Matrigel gels.

3.2.2.2 Frequency sweeps

A frequency sweep in the range $5*10^{-3}$-10 Hz was run on both samples at a temperature of 37 ºC immediately after the oscillation strain control measurements. The frequency sweeps were conducted at a fixed strain of $5*10^{-3}$, which was shown to be within the linear viscoelastic region (Figure 3-15 and Figure 3-16, strain sweeps). Results from the frequency sweeps are presented in Figure 3-13 and Figure 3-14.

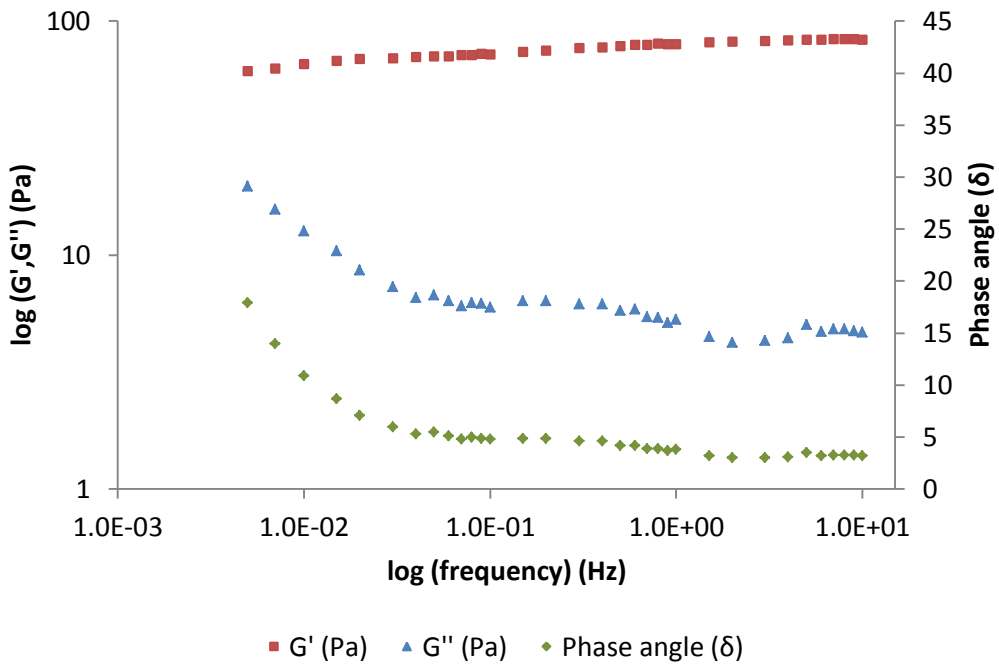

Figure 3-13. Frequency sweep of Matrigel from temperature ramp in the frequency range $5*10^{-3}$-10 Hz and at a fixed strain of $5*10^{-3}$. Measurements were run at a temperature of 37 ºC.

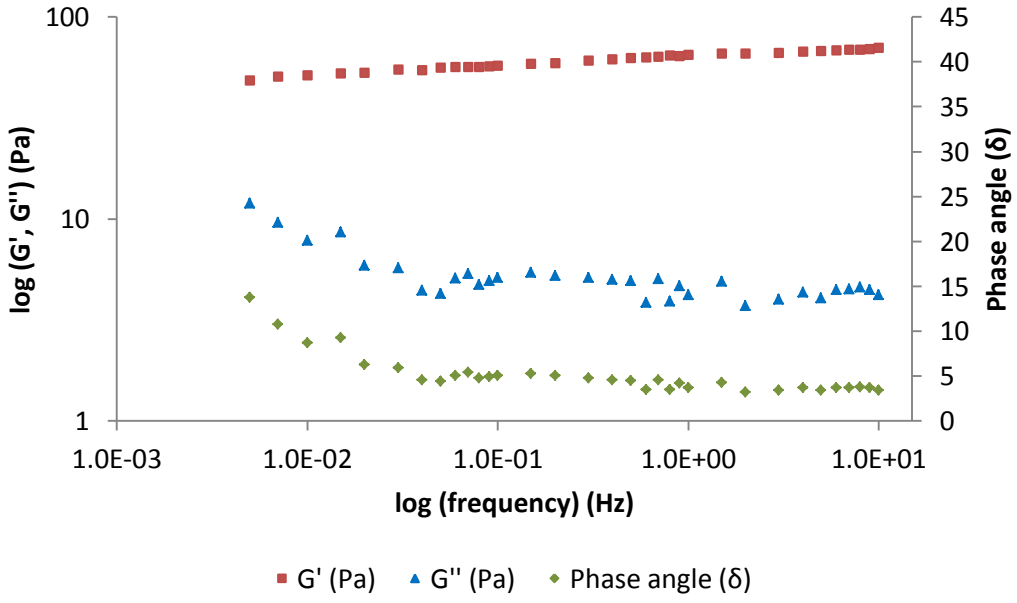

Figure 3-14. Frequency sweep of Matrigel from temperature gradient in the frequency range $5*10^{-3}$-10 Hz and at a fixed strain of $5*10^{-3}$. Measurements were performed at a temperature of 37 ºC.

Both frequency sweeps demonstrate a solid-like behaviour of Matrigel in the frequency range of $5*10^{-3}$-10 Hz, as the phase angle is below 20º. A reduction in the phase angle is observed as the frequency is increased, suggesting frequency dependence. Matrigel from the temperature ramp exhibit a more frequency dependent behaviour than Matrigel from the temperature gradient, as the change in phase angle during the sweep is larger and more pronounced. This suggests a network with more entanglements than in the network of the temperature gradient Matrigel, reflecting the less optimal gel setting caused by the temperature ramp. As suggested previously, the gel system in the temperature ramp was set before the most favorable interactions were allowed to occur, resulting in a gel system with less true gel properties and more entanglements than the temperature gradient Matrigel.

3.2.2.3 Strain sweeps

Frequency sweeps were immediately followed by strain sweeps in the strain region $1*10^{-4}$-1 and at a fixed frequency of 1 Hz to determine the viscoelastic region. Strain sweeps of Matrigel samples from the temperature ramp and the temperature gradient are presented in Figure 3-15 and Figure 3-16, respectively. Data points at strains 0.0001-0.001 are not presented in both strain sweeps because of low torque, resulting in a poor signal-to-noise ratio.

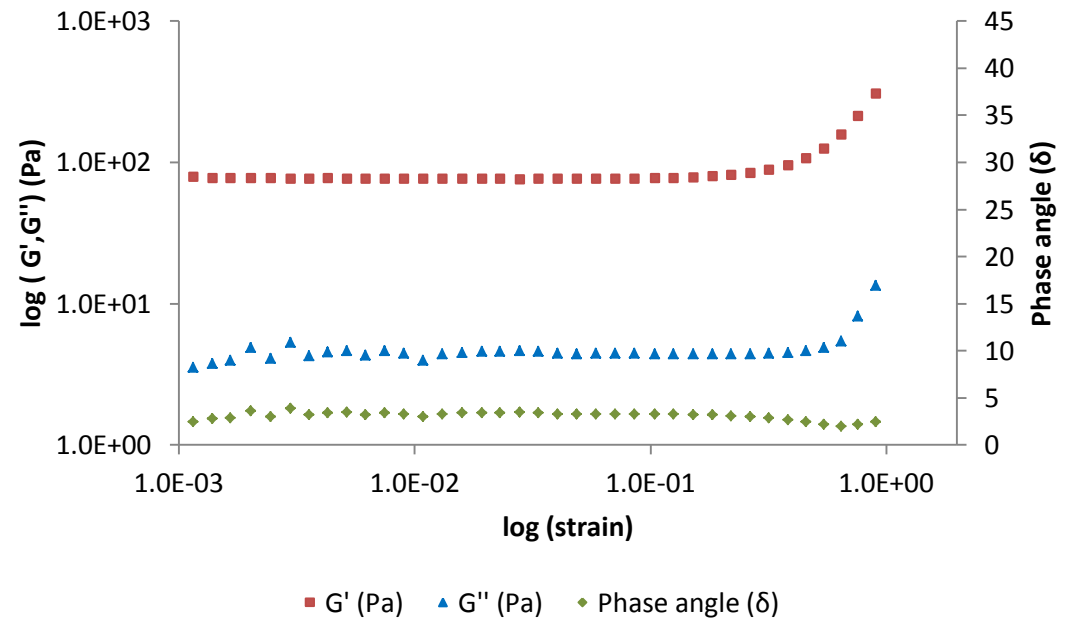

Figure 3-15. Strain sweep of Matrigel from temperature ramp in the range $1*10^{-4}$-1. Measurements were run at a frequency of 1 Hz and a temperature of 37 ºC.

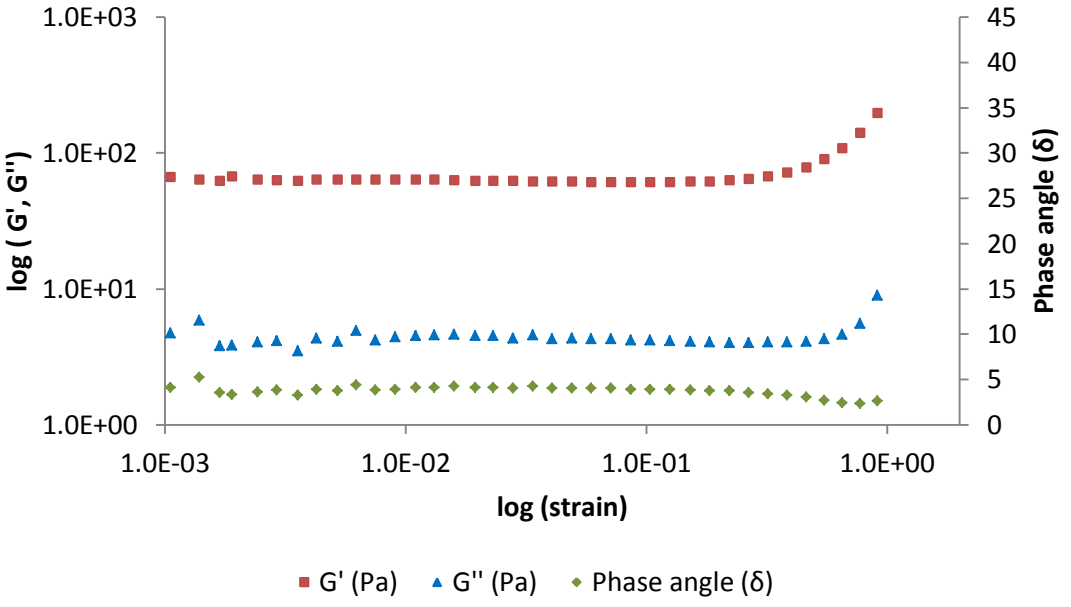

Figure 3-16. Strain sweep of Matrigel from temperature gradient in the range $1*10^{-4}$-1. Measurements were run at a frequency of 1 Hz and a temperature of 37 ºC.

Both strain sweeps presented in Figure 3-15 and Figure 3-16 demonstrate a clear linear viscoelastic region at strains 0.001 – 0.5. Within this region, the values of G', G'' and the phase angle are stable. The results show that the applied strain of $5*10^{-3}$ in the oscillation strain control measurements and frequency sweeps was within the LVR, in which the loss modulus (G'') and the storage modulus (G') are independent of the applied strain (Zhong and Daubert, 2013). The material exhibited strain dependence at strains higher than 0.5 with an increase in G' and G'' in both experiments. The phenomenon of increased gel strength can often be observed before a material is destroyed, as the elastic segments within the material is stretched to their limit before the interactions, entanglements and bonds of the network break.

3.2.3 Control rheological measurements of Matrigel diluted with saline

All diffusion experiments performed in this thesis utilized the same total volume of added compounds to Matrigel in the preparation of gels, thus the same degree of diluted Matrigel. Control rheological measurements of Matrigel diluted with saline were therefore conducted. A solution of Matrigel (450 µl) and saline (150 µl) was prepared according to section 2.2.1.4 Final protocol; Preparation of Matrigel gels, although the mixture was added directly to the plate of the rheometer instead of being incubated. Saline replaced the volume of G-block and model compound added to Matrigel in diffusion experiments described in section 3.1 Diffusion experiments.

3.2.3.1 Oscillation strain control

Gelling kinetics of diluted Matrigel were monitored by oscillation strain control measurements at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 39 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements. The setting curve of the gel solution is presented in Figure 3-17.

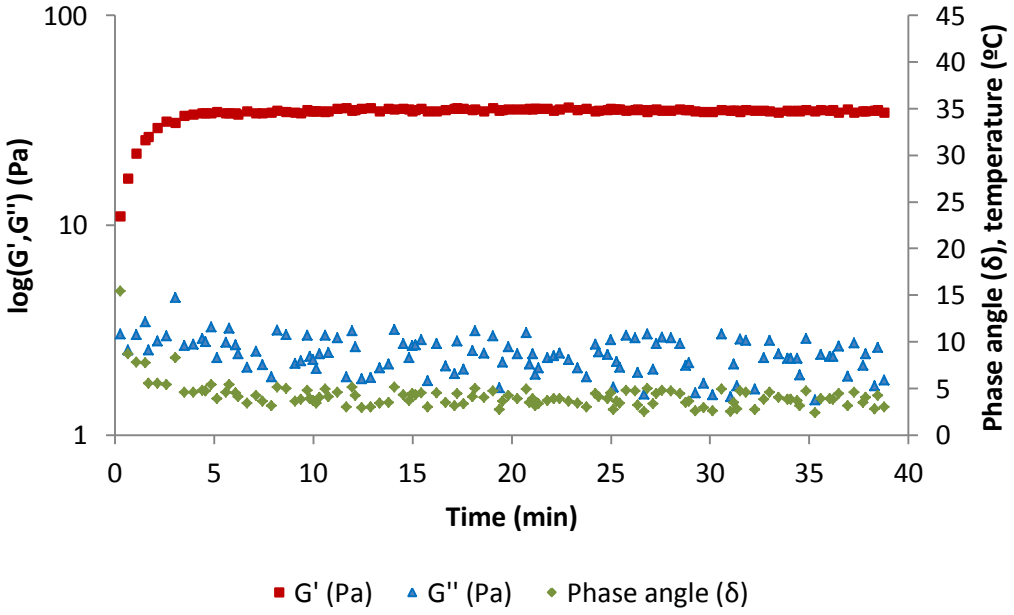

Figure 3-17. Setting curve of Matrigel diluted with saline. Measurements were run at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 39 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements and kept at 37 ºC during measurements.

As demonstrated in Figure 3-17, G' increases and levels off at 35 Pa as an equilibrium is obtained after 8 minutes. The observed phase angle values are under 20º, indicating a viscoelastic solid character. The characteristics of the curve resemble those of the setting curve of the Matrigel temperature ramp. The main difference is that the diluted Matrigel demonstrate a lower value of G' at equilibrium (35 Pa) compared to the undiluted Matrigel (90 Pa). The reduction in gel strength is expected as the gel network is diluted.

A similar experiment was performed prior to this experiment. The resulting setting curve showed a G' equilibrium value over a decade higher (700 Pa) than the presented curve in Figure 3-17. It was assumed that an error with the instrumental settings had occurred, possibly regarding the zero gapping.

3.2.3.2 Frequency sweep

A frequency sweep in the range 0.02-10 Hz was run on the diluted Matrigel directly after the oscillation strain control measurements. The frequency range was reduced from $5*10^{-3}$-10 Hz in previous conducted frequency sweeps due to no observed information of importance at the first sampling points. The frequency sweep was run at a temperature of 37 ºC and a set strain of $5*10^{-3}$, which was confirmed to be within the linear viscoelastic region (Figure 3-19, strain sweep). Results from the frequency sweep are displayed in Figure 3-18.

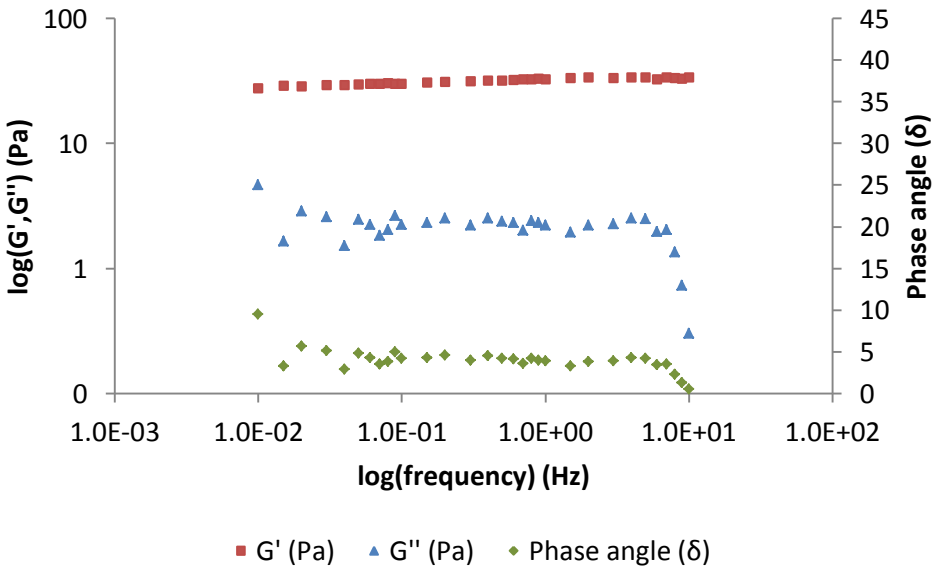

Figure 3-18. Frequency sweep of diluted Matrigel in the frequency range 0.02-10 Hz and at a fixed strain of $5*10^{-3}$. Measurements were run at a temperature of 37 ºC.

As presented in Figure 3-18, the diluted Matrigel responds in a frequency independent manner until the frequency of 6 Hz. Until this point, the system shows true gel properties with parallel G' and G'' and G' > G''. G'' and the phase angle drop in the frequency interval 6-10 Hz, suggesting frequency dependence in this region. The behaviour at higher frequencies might be a result of time-dependent entanglements, interactions and cross-links in the material causing the system to be observed with increased solid-like properties at short times of observation. By comparing this frequency sweep to the one for undiluted Matrigel with temperature ramp (Figure 3-14), the diluted Matrigel exhibit less frequency dependent behaviour than the undiluted Matrigel. This suggests that an effect of dilution is less entanglements and interactions between gelling components.

3.2.3.3 Strain sweep

A strain sweep was run immediately after the frequency sweep in the strain interval $1*10^{-4}$-100 to determine the linear viscoelastic region. The upper strain limit was increased from 1 in previous conducted strain sweeps to 100 in order to study the material during destruction. The strain sweep was performed at a set frequency of 1 Hz and a temperature of 37 ºC. Results from the strain sweep are presented in Figure 3-19. Data points at strains 0.0001-0.001 were omitted due to poor signal-noise ratio caused by low torque.

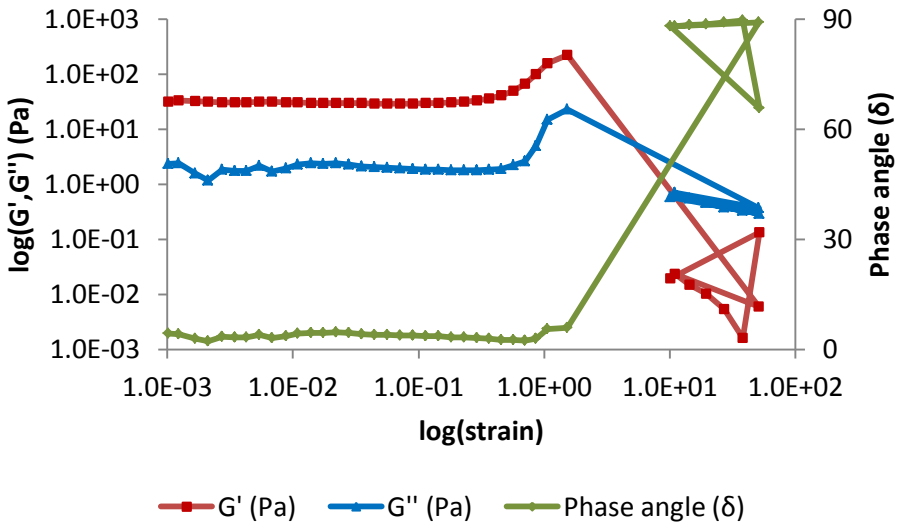

Figure 3-19. Strain sweep of diluted Matrigel from temperature ramp in the range $1*10^{-4}$-100. Measurements were run at a frequency of 1 Hz and a temperature of 37 ºC.

Figure 3-19 demonstrates a linear viscoelastic region with moduli independent of the applied strain in the strain region 0.001-0.5. The strain sweep show that the applied strain of $5*10^{-3}$ in the oscillation strain control measurements and frequency sweeps is within the LVR. The values of G' and G' increase before the gel network is destroyed at strain 1.5. When the bonds and interactions in the Matrigel is broken, the phase angle increases and a crossover between G' and G'' occurs as the material exhibit more liquid-like behaviour. The destroyed gel was visually observed as a collection of gel fragments.

The data points after the material destruction was included to illustrate the overshoot of applied strain by the rheometer immediately after destruction. The applied strain in each measurement is based on the previous measurement. Therefore, an overshoot arises as the instrument bases the applied strain after destruction on the strain imposed on the material prior to destruction.

3.2.4 The effect of G-block on the rheological properties of Matrigel

Rheological measurements of a solution of Matrigel and G-block (5 mg/ml, DP 12) were carried out in order to study the effect of G-block on the rheological properties of Matrigel. The gel solution was prepared according to section 2.2.1.4 Final protocol; Preparation of Matrigel gels and added to the rheometer plate.

3.2.4.1 Oscillation strain control

Gelling kinetics of Matrigel containing G-block (5 mg/ml) were monitored by oscillation strain control measurements at a set strain of $5*10^{-3}$ and frequency of 1 Hz for 314 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements. A similar experiment was performed before the presented one, with a duration of 75 minutes. The results showed an increase of G' to a maximum value of 54 Pa, followed by a reduction in G' without establishment of equilibrium. To investigate if G' leveled off and reached an equilibrium, the experimental duration was increased in the presented experiment. The setting curve is presented in Figure 3-20.

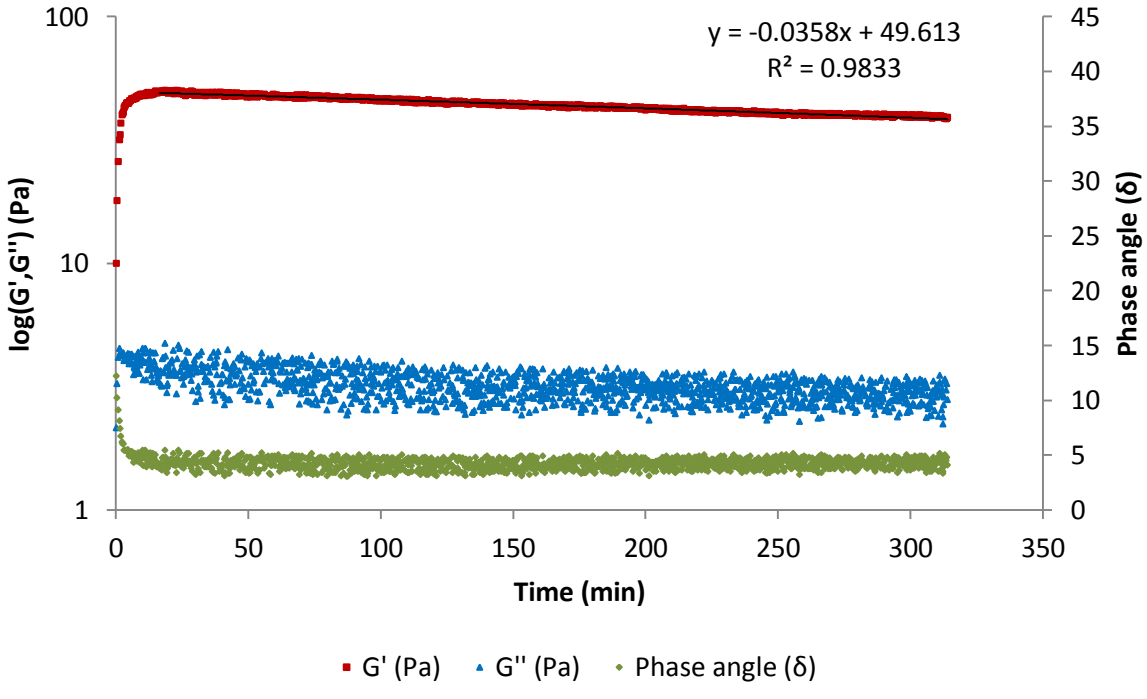

Figure 3-20. Setting curve of Matrigel with G-block (5 mg/ml). Measurements were run at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 314 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements, and kept at 37 ºC during measurements. A trend line describing the reduction of G' in the range of 16-314 minutes is given by the equation y = -0.0358x + 49.613.

Figure 3-20 demonstrates an increase in G' as the gel sets. G' reached a maximum value of 50 Pa after 20 minutes, before it decreased with 22 % to a value of 39 Pa after 314 minutes. A trend line of the G' values was made in the range of 16-314 minutes. The reduction of G' followed a linear trend with a slope of -0.0358 according to the trend line equation. The phase angle values were below 13º, indicating a solid-like behaviour throughout the experiment.

As observed, G' did not reach an apparent equilibrium in the timescale of the experiment. Therefore, one can question if an equilibrium would have been established if the duration of the experiment was longer. The setting curve does not show typical features of syneresis, as the reduction of G' follows a linear trend. If Matrigel does not exhibit syneresis, the reduction in G' suggests that G-block may reduce the elastic properties of Matrigel.

3.2.4.2 Frequency sweep

A frequency sweep was conducted on Matrigel containing G-block (5 mg/ml) immediately after the oscillation strain control measurements at a temperature of 37 ºC. The sweep was run in the frequency interval 0.02-10 Hz and at a fixed strain of $5*10^{-3}$, which was confirmed to be within the linear viscoelastic region (Figure 3-22, strain sweep). The results are illustrated in Figure 3-21.

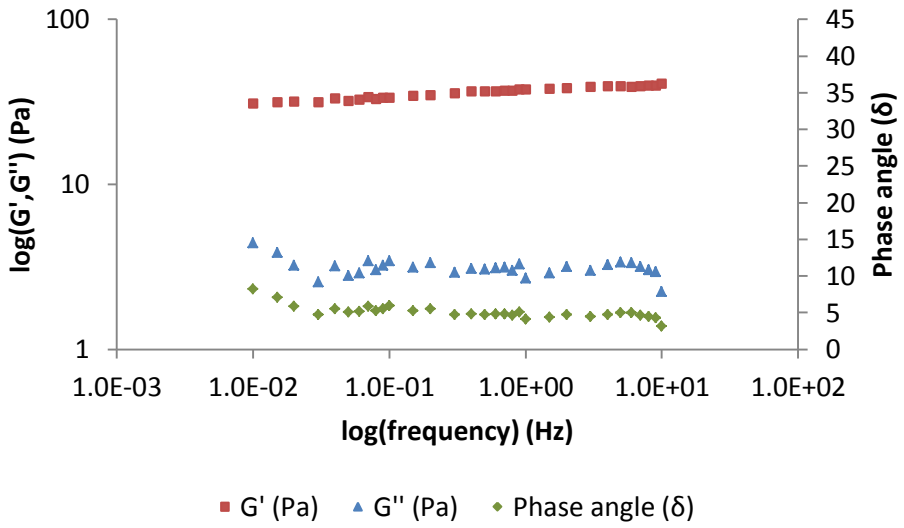

Figure 3-21. Frequency sweep of Matrigel with G-block (5 mg/ml) in the frequency range 0.02-10 Hz and at a fixed strain of $5*10^{-3}$. Measurements were run at a temperature of 37 ºC.

Figure 3-21 demonstrates a frequency independent behaviour of the Matrigel with G-block (5 mg/ml) in the frequency range of 0.02-10 Hz. The system shows true gel properties with G' > G'' and constant values of the moduli.

3.2.4.3 Strain sweep

The frequency sweep was directly followed by a strain sweep of Matrigel with G-block (5 mg/ml) in the strain region $1*10^{-4}$-100 and at a fixed frequency of 1 Hz. The strain sweep was conducted to determine the linear viscoelastic region. Strain sweep results are presented in Figure 3-22. Data points at strains 0.0001-0.001 were excluded from the plot due to low torque and poor signal-noise ratio.

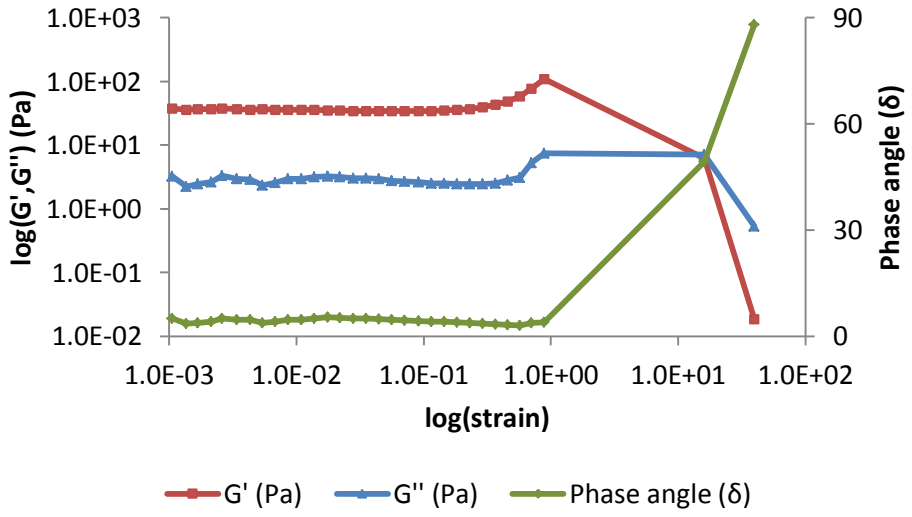

Figure 3-22. Strain sweep of Matrigel containing G-block (5 mg/ml) with increasing strain in the range $1*10^{-4}$-100. Run at a frequency of 1 Hz and a temperature of 37 ºC.

The strain sweep (Figure 3-22) shows a clear linear viscoelastic region in the strain interval 0.001-0.5. The results demonstrate that the applied strain of $5*10^{-3}$ in the oscillation strain control measurements and frequency sweeps is within the LVR. At strains higher than 0.5, the bonds and interactions in Matrigel breaks and the material is destroyed at strain 0.8. The phase angle increases dramatically as the network is disrupted and the material exhibit high viscoelastic liquid character.

3.2.5 Control rheological measurements of Matrigel containing tartrazine

Control rheological measurements were run on a gel solution of Matrigel and tartrazine (1 mM) to investigate whether tartrazine was affecting the rheological profile of Matrigel. The same gel solution was utilized in the gels for control diffusion experiments with tartrazine (1 mM). The solution was prepared according to section 2.2.1.4 Final protocol; Preparation of Matrigel gels before it was added to the rheometer plate.

3.2.5.1 Oscillation strain control

The gelling kinetics of Matrigel with tartrazine (1 mM) were monitored by oscillation strain control measurements at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 79 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements and stabilized at 37 ºC during measurements. The resulting setting curve is displayed in Figure 3-23.

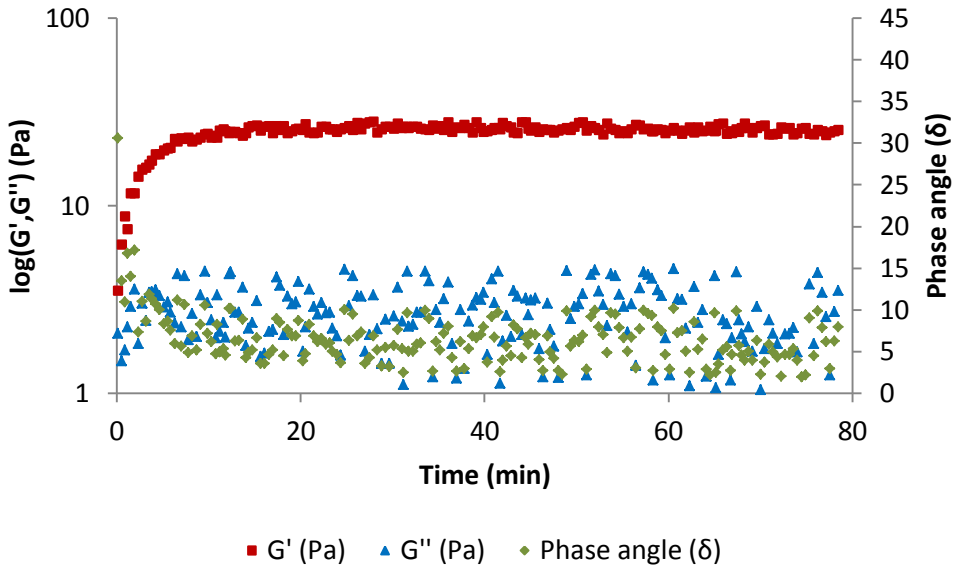

Figure 3-23. Setting curve of Matrigel with tartrazine (1 mM). Measurements were conducted at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 79 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements, and kept at 37 ºC during measurements.

Figure 3-23 demonstrates the establishment of an equilibrium after 15 minutes with G' levelled off at 26 Pa. The phase angle values are mainly below 20º, indicating a solid-like behaviour of the material. The setting curve resembles that of undiluted Matrigel from the temperature ramp (section 3.2.2). However, the value of G' at equilibrium for Matrigel containing tartrazine (1 mM) was lower than the undiluted Matrigel (90 Pa). The decrease in gel strength might be caused by the effect of dilution, as Matrigel was diluted when adding tartrazine. Influence of tartrazine on the gel network can also be a possibility. But since the value of G' at equilibrium (26 Pa) was close to the value of G' in Matrigel diluted with saline (35 Pa), the effect of dilution is suggested as the main explanation.

3.2.5.2 Frequency sweep

The oscillation strain control measurements were followed by a frequency sweep of Matrigel with tartrazine (1 mM) in the region 0.02-10 Hz. The frequency sweep was run at a fixed strain of $5*10^{-3}$ and at a temperature of 37 ºC. The results are presented in Figure 3-24.

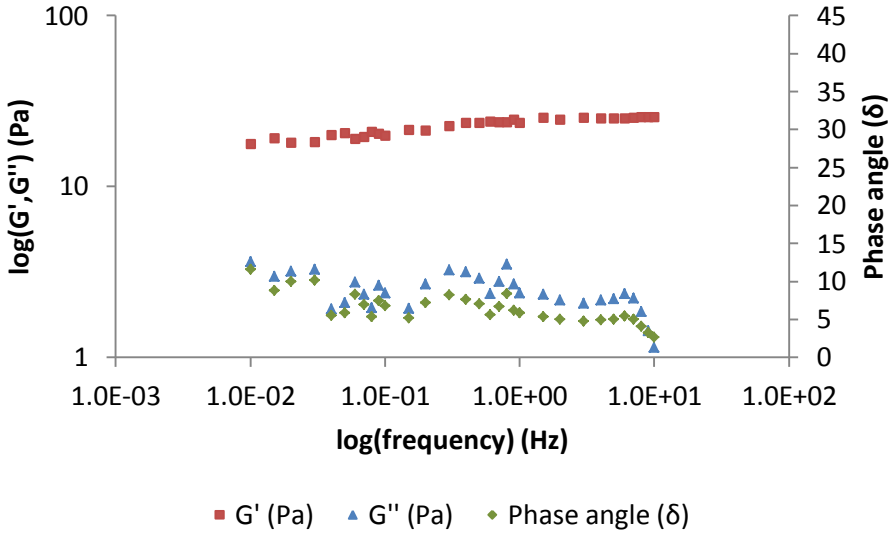

Figure 3-24. Frequency sweep of Matrigel containing tartrazine (1mM) in the frequency range 0.02-10 Hz and at a fixed strain of $5*10^{-3}$. Measurements were run at a temperature of 37 ºC.

The frequency sweep (Figure 3-24) demonstrates frequency independence of the Matrigel containing tartrazine (1 mM) as the ratio between G' and G'' is constant. The phase angle values are below 12º, indicating a system with true gel properties. The phase angle drops slightly from the frequency of 6 Hz, suggesting a small portion of time-dependent entanglements in the material.

3.2.5.3 Strain sweep

A strain sweep of Matrigel with tartrazine (1mM) in the strain interval $1*10^{-4}$-100 was run immediately after the frequency sweep to determine the linear viscoelastic region. Measurements were performed at a set frequency of 1 Hz and temperature of 37 ºC. The results are presented in Figure 3-25. Data points at strains 0.0001-0.002 are not presented due to poor signal-noise ratio caused by low torque.

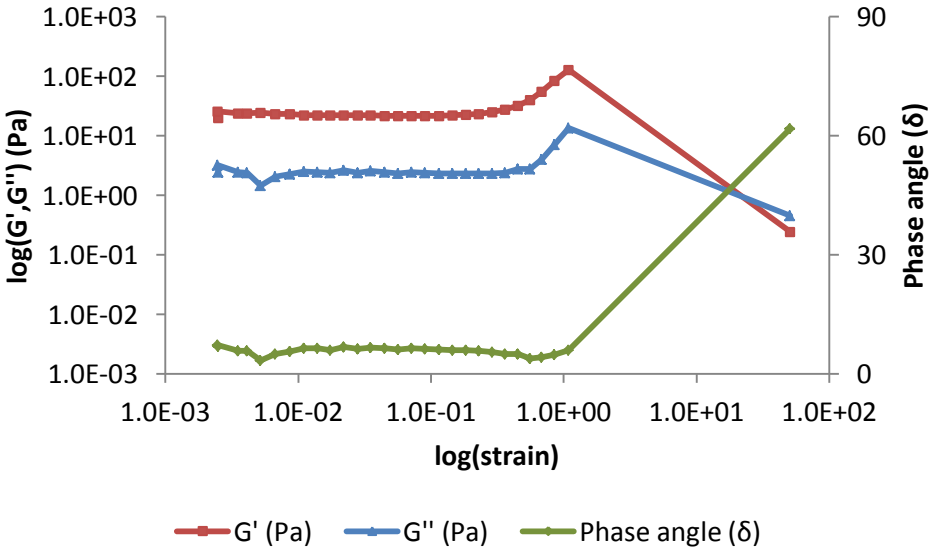

Figure 3-25. Strain sweep of Matrigel with tartrazine (1 mM) in the strain range of $1*10^{-4}$-100, run at a frequency of 1 Hz and a temperature of 37 ºC.

Figure 3-25 demonstrates a linear viscoelastic region in the strain region 0.002-0.5 with moduli independent of the applied strain. The strain sweep confirms that the applied strain of $5*10^{-3}$ in the oscillation strain control measurements and frequency sweeps is within the LVR. The gel strength increases from strain 0.5-1.1, before the various interactions and bonds break and the network is destroyed.

3.2.6 The effect of G-block on the rheological profile of Matrigel with tartrazine

Rheological measurements were run on a solution of Matrigel with G-block (5 mg/ml) and tartrazine (1 mM) in order to investigate the rheological properties of a gel with a G-block concentration found to have the largest effect on diffusion of tartrazine in previous conducted diffusion experiments (section 3.1.3). The solution was prepared according to section 2.2.1.4 Final protocol; Preparation of Matrigel gels followed by addition to the rheometer plate.

3.2.6.1 Oscillation strain control

Oscillation strain control measurements of Matrigel containing G-block (5 mg/ml) and tartrazine (1 mM) were conducted to study the gelling kinetics. Measurements were run at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 127 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements. The resulting setting curve of the gel solution is presented in Figure 3-26.

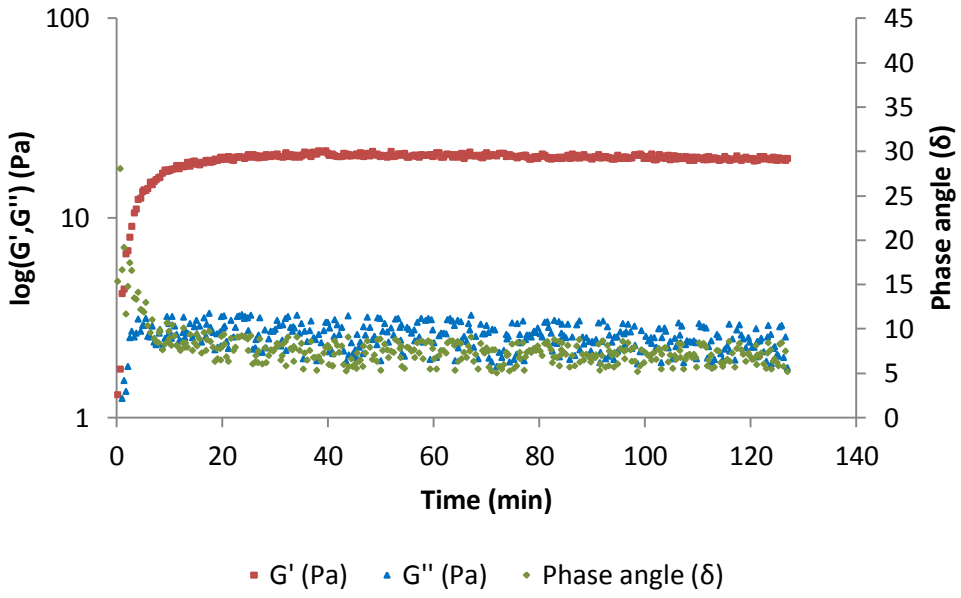

Figure 3-26. Setting curve of Matrigel containing G-block (5 mg/ml) and tartrazine (1 mM). Measurements were conducted at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz for 127 minutes. The temperature was ramped from 4 ºC to 37 ºC prior to measurements, and kept at 37 ºC during measurements.

The setting curve in Figure 3-26 demonstrates establishment of an equilibrium after 20 minutes with a G' value of 22 Pa. Matrigel with tartrazine (1 mM) and G-block (5 mg/ml) exhibited viscoelastic solid-like properties as the phase angle values were mainly below 20º throughout the experiment.

In the results of the setting curve of Matrigel with G-block (5 mg/ml) (section 3.2.4), G' decreased after gel setting and no equilibrium was achieved. It was therefore expected to observe a similar trend in this experiment, with the assumption that tartrazine is not affecting the gel network. As illustrated in Figure 3-26, no reduction in G' after gel setting was observed as an equilibrium was obtained. However, the value of G' at equilibrium (22 Pa) was the lowest observed among the tested gels in this thesis, suggesting that G-block affected the Matrigel network during gel setting. The results do not provide enough support for this hypothesis, as the G' value in this experiment was close to the G' values for Matrigel diluted with saline and tartrazine (1 mM).

3.2.6.2 Frequency sweep

A frequency sweep was run on Matrigel with G-block (5 mg/ml) and tartrazine (1 mM) in the range 0.02-10 Hz after the oscillation strain control measurements. The sweep was performed at a temperature of 37 ºC and at a fixed strain of $5*10^{-3}$, which was confirmed to be within the linear viscoelastic region (Figure 3-28, strain sweep). The results are presented in Figure 3-27.

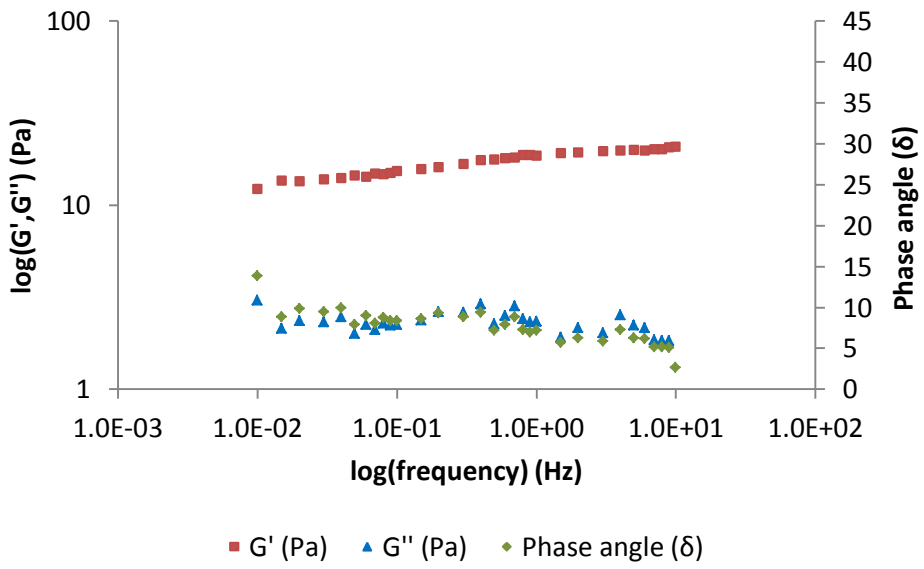

Figure 3-27. Frequency sweep of Matrigel containing G-block (5 mg/ml) and tartrazine (1mM) in the frequency range 0.02-10 Hz and at a fixed strain of $5*10^{-3}$. Measurements were run at a temperature of 37 ºC.

Figure 3-27 demonstrates parallel G' and G'' with G' > G'', suggesting a frequency independent behaviour of Matrigel containing G-block (5 mg/ml) and tartrazine (1 mM). The system shows true gel properties with phase angle values below 15º.

3.2.6.3 Strain sweep

The frequency sweep was directly followed by a strain sweep of Matrigel containing G-block (5 mg/ml) and tartrazine (1 mM) in the range $1*10^{-4}$-100. The strain sweep was conducted to determine the linear viscoelastic region and was run at a temperature of 37 ºC and a fixed frequency of 1 Hz. The results are presented in Figure 3-28. Data points at strains 0.0001-0.001 are not presented in the plot due to low torque and poor signal-noise ratio.

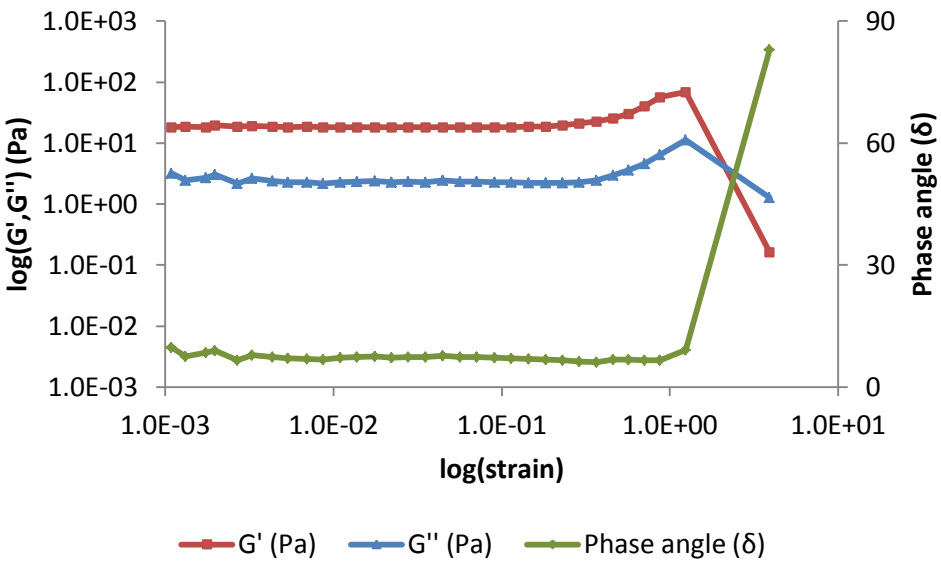

Figure 3-28. Strain sweep of Matrigel with G-block (5 mg/ml) and tartrazine (1 mM) in the strain range of $1*10^{-4}$-100. Run at a frequency of 1 Hz and a temperature of 37 ºC.

A linear viscoelastic region is observed at strains 0.001-0.5 in the strain sweep (Figure 3-28). The applied strain of $5*10^{-3}$ in the oscillation strain control measurements and frequency sweeps was therefore within the LVR. Matrigel with G-block (5 mg/ml) and tartrazine (1 mM) show a strain dependent behaviour at strains higher than 0.5 and the gel network is destroyed at strain 1.2.

3.2.7 Summary rheological characterization of Matrigel

An overview of the composition of the different gels rheologically tested in this thesis and their maximal value of storage modulus (G') during oscillation strain control measurements is presented in Table 3-18. Information about whether the gels reached equilibrium during the measurements is also included.

Table 3-18. Overview of gel composition and their respective G' max values from oscillation strain control measurements performed at a fixed strain of $5*10^{-3}$ and frequency of 1 Hz. The temperature was rapidly increased from 4 ºC to 37 ºC prior to measurements, and kept at 37 ºC during measurements.

Gel	Composition	G' max (Pa)	Apparent equilibrium?
1	Matrigel (temperature ramp)	90	Yes
2	Matrigel, saline	35	Yes
3	Matrigel, G-block (5 mg/ml)	50	No. G' decreased
4	Matrigel, tartrazine (1 mM)	26	Yes
5	Matrigel, G-block (5 mg/ml), tartrazine (1 mM)	22	Yes

The rheological profile of Matrigel was characterized by two methods; temperature gradient and temperature ramp. Matrigel from the temperature gradient exhibited syneresis, whereas the temperature ramp resulted in a setting curve more suitable as a basis for comparison. In addition, the temperature ramp method had the advantage of resemble the preparation method of gels utilized in the diffusion experiments. The temperature ramp method was therefore utilized in the rheological characterization of Matrigel with different added components. The setting curves of Matrigel with added saline and added tartrazine (1 mM) demonstrated establishment of equilibrium with G' values of 35 Pa and 26 Pa, respectively. The reduction in gel strength was assumed to be caused by the dilution of the gel network.

Matrigel containing G-block (5 mg/ml) did not reach an apparent equilibrium in the timescale of the experiment, as storage modulus (G') continuously decreased after gel setting. Due to a linear reduction of G', the possibility of syneresis was disregarded. The phenomenon of G' reduction was not observed in the other gels tested, suggesting an effect of G-block on Matrigel. This is an interesting result, which may be linked to the observed increase of model

compound diffusion from Matrigel with G-block in the previous described diffusion experiments (section 3.1). The same trend of linear reduction in G' was not observed in the setting curve of Matrigel with G-block (5 mg/ml) and tartrazine (1 mM), in which an equilibrium was established with a G' value of 22 Pa. This was the lowest G' equilibrium value observed among the gels tested. The results suggested an effect of G-block during gel setting. Based on the results from the experiments with G-block, it was suggested that G-block can affect the elastic properties of Matrigel. The observed decrease in G' in Matrigel with G-block (5 mg/ml) was particularly interesting. However, by comparing the G' values in Table 3-18, G-block did not exhibit a larger effect on the rheological properties of Matrigel than saline and tartrazine (1 mM).

3.3 General discussion

An increasing number of studies support the importance of the tumour microenvironment in tumour progression. Many types of cancers associated with high mortality rates, such as pancreatic cancer, develop an extensive fibrotic extracellular matrix (Olson and Hanahan, 2009). Over-expression of ECM may result in an increased barrier to drug diffusion and inefficient tumour drug delivery (Sriraman *et al.*, 2014). Furthermore, a correlation between ECM stiffness and macromolecule diffusion resistance in the tumour interstitium has also been reported (Netti *et al.*, 2000).

Studies have demonstrated the ability of G-block to alter the mucus barrier function and matrix structure (Nordgard and Draget, 2011, Taylor Nordgård *et al.*, 2014). Improved nanoparticle mobility, increased matrix pore size and a reduced density of network cross-links have been reported after addition of guluronate oligomers to mucus matrices (Taylor Nordgård *et al.*, 2014). It is suggested that the alterations in mucus barrier properties and structure are caused by G-block inhibiting electrostatic interactions within the mucus matrix. Another theory suggests that guluronate oligomers inhibit interactions between matrix mucins and the mobile component. Furthermore, the alteration in barrier properties could also be due to inhibition of matrix cross-links, resulting in a reduction in the steric barrier (Draget and Taylor, 2011, Taylor Nordgård *et al.*, 2014). G-block is currently under investigation for the potential application in cancer treatment under the name of RiXOVA. Experimental *in vivo* data has shown that G-block reduces tumour growth in a mouse model of pancreatic cancer, both as a single agent and in combination with the standard cytostatic gemcitabine. However, the mechanism of action of G-block remains to be explained. A hypothesis is that in tumours with an over-expression of extracellular matrix, such as pancreatic cancer, G-block can reduce the barrier function of the extracellular matrix in a similar way as observed in mucus matrices, resulting in improved drug and immune system access to the cancer cells.

This thesis has demonstrated that G-block increases the diffusion of tartrazine and IgG from Matrigel, which is a reconstituted basement membrane extracted from EHS tumours in mice. The results indicate that Matrigel is affected by G-block. This is supported by rheological data, which demonstrated a reduction in the elastic properties of Matrigel after addition of G-block. The results indicated that G-block is able to alter the network structure of Matrigel and

reduce the barrier function. However, it is important to emphasize that most of the experiments have not been repeated for verification due to time limitations.

4. Conclusion

The development and optimization of a diffusion experiment utilizing Matrigel as model for extracellular matrix was successful. By utilizing this method, diffusion of the model compounds tartrazine and IgG from Matrigel with and without G-block was investigated. G-block demonstrated an increased diffusion rate of both tartrazine and IgG from Matrigel. The results of testing a range of G-block concentrations suggested the effect of G-block to be concentration dependent. The mechanism of how G-block affects the diffusion of tartrazine and IgG in Matrigel is most likely complex and remains to be understood. In this thesis, a theory that G-block affects a bound fraction of tartrazine and IgG in Matrigel by binding competitively to the gel was hypothesized. In addition, it was postulated that G-block can reduce the steric hindrance in Matrigel by affecting the structure of the gel network. However, due to the complex nature of the Matrigel system, an explanation of the interactions between Matrigel and G-block is most likely multifactorial.

Gels utilized in the diffusion experiments were rheologically characterized to further investigate the effect of G-block on the rheological properties of Matrigel. G-block did not demonstrate an effect on the rheological profile of Matrigel compared to saline and tartrazine. However, the characterization of Matrigel containing only G-block demonstrated an interesting result. In contrast to the other gels, the gel did not reach an apparent equilibrium as storage modulus (G') decreased linearly after gel setting. The rheological data suggest that G-block may reduce the elastic properties of Matrigel, which might be linked to the observed increase in diffusion rate of tartrazine and IgG from Matrigel in the diffusion experiments. However, more work is needed to verify the presented results.

Matrigel is a reconstituted basement membrane extracted from EHS tumours in mice. This model system will to some extent resemble the ECM in tumour tissue *in vivo*. However, limitations include that Matrigel is a basement membrane and not connective tissue ECM. As the majority of cancer tumours is localized in tissues, the composition and structure of ECM will be different from that in a basement membrane. In addition, the structure of extracellular matrix varies among different cancer types. Furthermore, the conditions will be different and more complex *in vivo* than in the model system utilized in this thesis.

5. Future work

In this thesis, diffusion experiments demonstrated an increased diffusion rate of tartrazine and IgG from Matrigel with G-block. The diffusion experiments results indicated a concentration dependent effect of G-block. Therefore, it would have been interesting to perform diffusion experiments with higher concentrations of G-block to investigate if the effect increased or reached a maximum level.

Results from the rheological measurements demonstrated a reduction in the elastic properties of Matrigel with G-block after gel setting. The effect of G-block on the mechanical properties of Matrigel could be investigated further by performing rheological measurements for a longer period of time to find out whether an equilibrium is established. Conducting rheological measurements on Matrigel using a wider range of G-block concentrations could also provide more information about the effect of G-block. In addition, it would be interesting to study the network structure of Matrigel with and without G-block by utilizing scanning electron microscopy. Future work could also include studies of the mobility of model compounds in Matrigel with G-block by using fluorescence recovery after photobleaching (FRAP).

More studies are required to make any conclusions about the effect of G-block on the extracellular matrix barrier to drug diffusion. It would have been interesting to study molecular diffusion in other model systems than Matrigel, as different types of extracellular matrix vary in composition and structure, which may in turn affect molecular diffusion.

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List of appendices

Appendix A: Matrigel protocol

CERTIFICATE OF ANALYSIS

PRODUCT: Corning® Matrigel® Basement Membrane Matrix, 10 ml vial
CATALOG NUMBER: 354234

LOT NUMBER: 3340868

SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor
FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin
Corning Matrigel Basement Membrane Matrix is compatible with all culture media
STORAGE: Store at -20°C. Avoid multiple freeze-thaws. Do not store in frost-free freezer. **KEEP FROZEN.**

QUALITY CONTROL:

Specification	Criteria	Result
Protein Concentration	Results obtained by Lowry method and represented in mg/ml.	8.4
Endotoxin	Endotoxin units (EU)/ml are measured by Limulus Amoebocyte Lysate assay.	6.28
Gelling	Tested for ability to gel quickly and maintain this form with culture medium for a period of 14 days at 37°C.	PASS
Biological Activity	Biological activity is determined using a neurite outgrowth assay. Chick dorsal root ganglia are plated on a 1.0 mm layer of Corning Matrigel Matrix. Tested for a positive neurite outgrowth response after 48 hours without addition of nerve growth factor.	PASS
Sterility	Tested for the presence of bacteria, fungi and mycoplasma.	NEGATIVE
MAP Test	Mouse colonies screened for Sendai, MHV, PVM, TEMV/GDVII, Ectro, Polyoma, MRV/EDIM, LCM, MCMV, M.Ad, Reo, MPV, LDEV/LDHV, MTV, Hantaan, K, RCMV, CARB	NEGATIVE
PCR Test	Tumor source tested for *Mycoplasma spp.*, *Helicobacter*, LDEV/LDHV, Sendai, MHV, PVM, MMV/MVM, MPV, Reo (1, 2, 3), MRV/EDIM, Ectro, LCM, K, MTV, Polyoma, Hantaan, Seoul, M. Ad (1, 2), MCMV, Norovirus, TMEV/GDVII, KRV, Toolan's H-1, RCV/SDA Finished goods tested for LDEV/LDHV.	NEGATIVE

Expiration Date: February 29, 2016

Quality Assurance _____

Date _____

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GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Basement Membrane Matrix, 10 mL vial

CATALOG NUMBER: 354234

BACKGROUND:

Basement membranes are thin extracellular matrices underlying cells *in vivo*. Corning Matrigel Basement Membrane Matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins. Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycans, entactin/nidogen.[1,2] Corning Matrigel Basement Membrane Matrix also contains TGF-beta, epidermal growth factor, insulin-like growth factor, fibroblast growth factor, tissue plasminogen activator,[3,4] and other growth factors which occur naturally in the EHS tumor. Corning Matrigel Basement Membrane Matrix is effective for the attachment and differentiation of both normal and transformed anchorage dependent epithelioid and other cell types. These include neurons,[5,6] hepatocytes,[7] Sertoli cells,[8,9] chick lens,[10] and vascular endothelial cells.[11] Corning Matrigel Basement Membrane Matrix will influence gene expression in adult rat hepatocytes,[12,13] vascular endothelial cells,[14] as well as three dimensional culture in mouse[15-18] and human[19,20] mammary epithelial cells. It is the basis for several types of tumor cell invasion assays,[21,22] will support *in vivo* peripheral nerve regeneration,[23-25] and provides the substrate necessary for the study of angiogenesis both *in vitro*[26,27] and *in vivo*.[25, 28-30] Corning Matrigel Basement Membrane Matrix also supports *in vivo* propagation of human tumors in immunosupressed mice.[31-33] Corning Matrigel Basement Membrane Matrix can be used for the transplantation of unsorted mammary cells,[34] as well as sorted epithelial subpopulations embedded in Corning Matrigel.[35,36] This matrix has also been used as a cancer stem cell model and shown to enhance tumor growth rates *in vivo*.[37]

SOURCE:

Engelbreth-Holm-Swarm (EHS) Mouse Tumor

FORMULATION:

Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.
Corning Matrigel Basement Membrane Matrix is compatible with all culture media.

STORAGE:

Stable when stored at -20°C. Freeze thaws should be minimized by aliquotting into one time use aliquots. Store aliquots in the -20°C freezer until ready for use. **DO NOT STORE IN FROST-FREE FREEZER. KEEP FROZEN.**

EXPIRATION DATE:

The expiration date for Corning Matrigel Basement Membrane Matrix is lot specific and can be found on the product Certificate of Analysis.

CAUTION:

It is extremely important that Corning Matrigel Basement Membrane Matrix and all cultureware or media coming in contact with Corning Matrigel Basement Membrane Matrix should be pre-chilled/ice-cold since Corning Matrigel Basement Membrane Matrix will start to gel above 10°C. Keep Corning Matrigel Matrix on ice at all times.

RECONSTITUTION AND USE:

Color variations may occur in frozen or thawed vials of Corning Matrigel Basement Membrane Matrix, ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. Variation in color is normal, does not affect product efficacy, and will disappear upon equilibration with 5% CO_2.

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Thaw Corning® Matrigel® Basement Membrane Matrix by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once Corning Matrigel Basement Membrane Matrix is thawed, swirl vial to ensure that material is evenly dispersed. Keep Corning Matrigel Matrix on ice at all times. Handle with sterile technique. Place thawed vial of Corning Matrigel Basement Membrane Matrix in sterile area, spray top of vial with 70% ethanol and air dry.

Corning Matrigel Basement Membrane Matrix may be gently pipetted using a pre-cooled pipet to ensure homogeneity. Aliquot Corning Matrigel Basement Membrane Matrix to tubes, switching tips whenever Corning Matrigel Basement Membrane Matrix is clogging the tip and/or causing the pipet to measure inaccurately. Gelled Corning Matrigel Basement Membrane Matrix may be re-liquified if placed at 4°C in ice for 24-48 hours.

Corning Matrigel Basement Membrane Matrix may be used as a thin gel layer (0.5 mm), with cells plated on top. Cells may also be cultured inside the Corning Matrigel Basement Membrane Matrix, using a 1 mm layer. Extensive dilution will result in a thin, non-gelled protein layer. This may be useful for cell attachment, but may not be as effective in differentiation studies.

COATING PROCEDURES:

Corning Matrigel Basement Membrane Matrix may be used in several ways. The Thin Gel Method is useful for plating cells on top of the gel, the Thick Gel Method allows you to grow cells within a three dimensional matrix, and the Thin Coating Method (no gel) provides you with a complex protein layer on top of which to grow your cells. Make your selection based on the final result that you wish to achieve, whether it is cell growth, attachment or differentiation.

NOTE: Application specific protocols are posted on the Corning support web page.* The protein concentration for Corning Matrigel Matrix products is lot specific and provided on the Certificate of Analysis. For consistent results dilute Corning Matrigel Matrix products by calculating the specific protein concentration (mg/mL) required. To maintain a gelled consistency we recommend not diluting Corning Matrigel Matrix to less than 3 mg/mL. Use ice-cold serum-free medium to dilute Corning Matrigel Matrix. Mix by pipetting up and down or by swirling the vial in ice.

Thin Gel Method

1. Thaw Corning Matrigel Basement Membrane Matrix as recommended. Using cooled pipets, mix the Corning Matrigel Basement Membrane Matrix to homogeneity.
2. Keeping culture plates on ice, add 50 µl/cm^2 of growth surface.
3. Place plates at 37°C for 30 minutes.
4. If necessary aspirate unbound material just before use and rinse gently using serum-free medium. Ensure that the tip of the pipet does not scratch the coated surface. Plates are now ready to use.

Thick Gel Method

1. Thaw Corning Matrigel Basement Membrane Matrix as recommended. Using cooled pipets, mix the Corning Matrigel Basement Membrane Matrix to homogeneity.
2. Keep culture plates on ice. Add cells to Corning Matrigel Basement Membrane Matrix and suspend using cooled pipets. Add 150-200 µl/cm^2 of growth surface.
3. Place plates at 37°C for 30 minutes. Culture medium may now be added. Cells may also be cultured on top of this gel.

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Thin Coating Method

1. Thaw Corning® Matrigel® Basement Membrane Matrix as recommended. Using cooled pipets, mix the Corning Matrigel Basement Membrane Matrix to homogeneity.
2. Dilute Corning Matrigel Basement Membrane Matrix to desired concentration using serum-free medium. Empirical studies should be completed to determine the optimal coating concentration for your application.
3. Add diluted Corning Matrigel Basement Membrane Matrix to vessel being coated. Quantity should be sufficient to cover entire growth surface easily. Incubate at room temperature for one hour.
4. Aspirate unbound material and rinse gently using serum-free medium. Plates are now ready to use.

CELL RECOVERY:

Corning Dispase (Cat. No. 354235), Corning Cell Recovery Solution (Cat. No. 354253).

Most efficient recovery of cells growing on Corning Matrigel Basement Membrane Matrix is accomplished using Corning Cell Recovery Solution that depolymerizes the Corning Matrigel Basement Membrane Matrix within 7 hours on ice or with Corning Dispase, a metalloenzyme which gently releases the cells allowing for continuous culture.

*NOTE: For technical resources please visit support page at **www.corning.com/lifesciences**

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Appendix B: Additional info to section 2 Materials and methods

B.1 Tartrazine gel concentration calculations

This appendix provides the calculations for the maximum and minimum tartrazine concentration in Matrigel.

First, a repetition of the diffusion experiment procedure:
Tartrazine is diffusing from Matrigel (80 µl) into the saline solution (600 µl) in the Transwell. A sample of 300 µl was removed from the saline solution and transferred to a cuvette at the time of sampling. Saline (300 µl) was added to the saline solution after each sampling to maintain the volume. The sample in the cuvette (300 µl) was diluted with saline (1500 µl or 1100 µl for absorbance cuvettes) until the optimal cuvette sample volume was reached (1800 µl or 1400 µl for absorbance cuvettes), before performing absorbance measurements.

B.1.1 Maximum tartrazine concentration in Matrigel

The maximum tartrazine concentration in Matrigel was calculated based on the maximum measurable tartrazine concentration in the final saline solution in the cuvette (0.03771 mM):

Cuvette sample volume: 1800 µl
Sample volume: 300 µl
Gel volume: 80 µl
Saline volume in Transwell: 600 µl

Tartrazine concentration in the saline solution in Transwell:
(0.03771 mM * 1800 µl)/300 µl = 0.2262 mM

Maximum tartrazine concentration in gel:
(0.2262 mM * 600 µl)/80 µl = **1.70 mM**

B.1.2 Minimum tartrazine concentration in Matrigel

The minimum tartrazine concentration in Matrigel was calculated based on the minimum measurable tartrazine concentration in the final saline solution in the cuvette (0.00314 mM):

Cuvette sample volume: 1800 µl
Sample volume: 300 µl
Gel volume: 80 µl
Saline volume in Transwell: 600 µl

Tartrazine concentration in the saline solution in Transwell:
(0.00314 mM * 1800 µl)/300 µl = 0.01884 mM

Maximum tartrazine concentration in gel:
(0.01884 mM * 600 µl)/80 µl = **0.14 mM**

B.2 Tartrazine standard curve (new cuvette type)

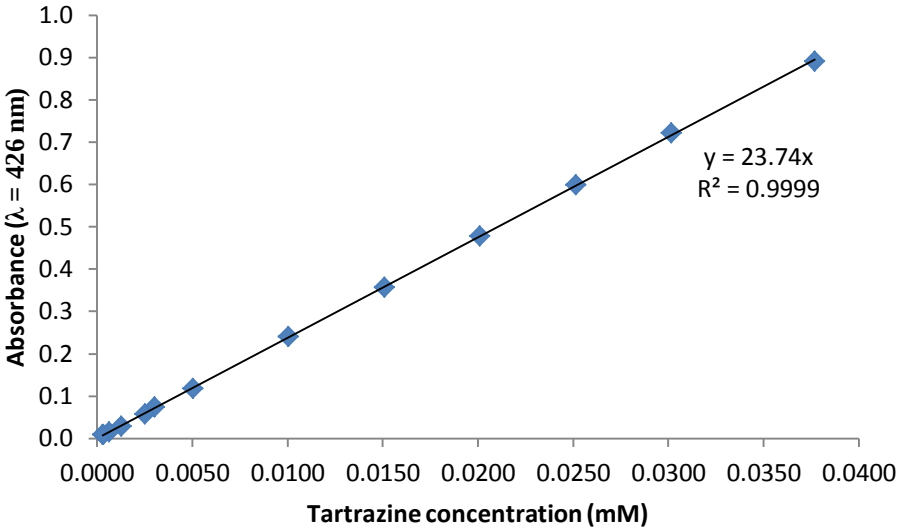

Figure B.2.1. Standard curve of tartrazine with a new cuvette type. Measurements were performed at wavelength 426 nm.

B.3 Alexa Fluor® 488 IgG standard curve

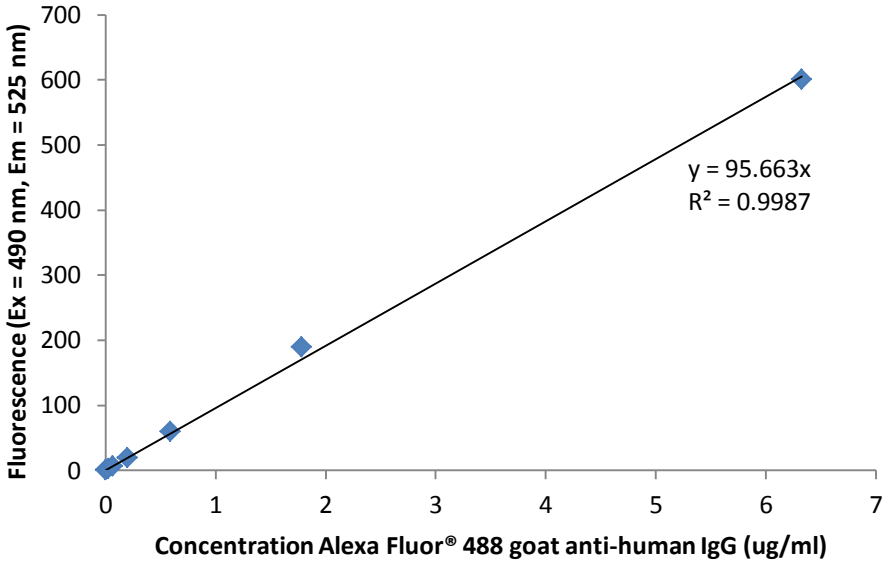

Figure B.3.1. Standard curve of Alexa Fluor® 488 IgG. Measurements were performed with a set excitation maximum of 490 nm and emission maximum of 525 nm.

Appendix C: Diffusion experiments

C.1 Diffused model compound concentration calculation example

The diffused tartrazine or IgG concentration at each time of sampling in the diffusion experiments was determined by absorbance or fluorescence measurements, followed by backwards calculations. The same calculation method was used in all diffusion experiments.

First, a repetition of the diffusion experiment procedure:
Tartrazine or IgG is diffusing from Matrigel (80 µl) into the saline solution (600 µl) in the Transwell. A sample of 300 µl was removed from the saline solution and transferred to a cuvette at the time of sampling. Saline (300 µl) was then added to the saline solution after each sampling to maintain the volume. The sample in the cuvette (300 µl) was diluted with saline (1500 µl or 1100 µl for absorbance cuvettes) until the optimal cuvette sample volume was reached (1800 µl or 1400 µl for absorbance cuvettes), before performing absorbance or fluorescence measurements.

The calculation method is described by using an example of a sample from a tartrazine diffusion experiment:

Measured absorbance: 0.1408

Standard curve equation: absorbance = 23.713 * concentration
Tartrazine concentration in cuvette: 0.1408/23.713 = 0.00594 mM

Total volume in cuvette (sample + saline): 1800 µl
Sample volume: 300 µl
Tartrazine concentration in sample before dilution:
(0.00594 mM *1800 µl)/300 µl = **0.03563 mM**

At the first point of sampling, the concentration of 0.03563 mM would equal the diffused tartrazine concentration. At later sampling points, the tartrazine concentration present in the saline solution from the last point of sampling would have to be subtracted. To create the

diffusion curve, the diffused tartrazine concentration at each time of sampling was added to the previous one.

Maximum diffused model compound concentration

The maximum diffused tartrazine or IgG concentration was calculated based on the assumption that the entire amount of model compound in Matrigel diffused into the saline solution. The calculations presented below include a Matrigel volume of 80 µl, saline solution volume of 600 µl and tartrazine concentration of 1 mM in Matrigel.

Maximum diffused tartrazine concentration from Matrigel into the saline solution:
(1 mM * 80 µl)/600 µl = 0.133 mM

This means that a concentration of 1 mM tartrazine in the gel corresponds to a maximum potential diffused tartrazine concentration of 0.133 mM in the saline solution. The percentage diffused tartrazine at each sampling point was calculated as a percentage of this value.

C.2 Diffusion experiments raw data

C.2.1 Diffusion experiment with tartrazine (1 mM) before optimization

Table C.2.1.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) in Matrigel.

	Absorbance				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
5	0.0569	0.0609	0.0587	0.0664	0.0686
20	0.0698	0.0889	0.0993	0.1113	0.1168
35	0.0676	0.0853	0.0872	0.0742	0.1002
50	0.0553	0.0621	0.075	0.0909	0.0832
65	0.0523	0.0679	0.0614	0.0742	0.0721
80	0.0375	0.0533	0.0512	0.0662	0.0699
95	0.0384	0.0491	0.049	0.0472	0.0614
110	0.0313	0.0379	0.0403	0.0545	0.0538
125	0.0151	0.0231	0.0215	0.0328	0.0367
140	0.0152	0.0187	0.0155	0.0238	0.0268
155	0.0039	0.0035	0.0106	0.014	0.0158
185	0.0161	0.0227	0.022	0.0284	0.0286
215	0.0263	0.0314	0.0274	0.0388	0.0396
245	0.0208	0.0269	0.026	0.033	0.0353
275	0.0192	0.0219	0.0233	-	0.0293
1346	0.0338	0.0409	0.0397	0.055	0.0527

Table C.2.1.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) in Matrigel.

	Diffused tartrazine concentration (mM)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
5	0.01440	0.01541	0.01485	0.01680	0.01736	0.0158	0.0013
20	0.02486	0.03020	0.03255	0.03656	0.03823	0.0325	0.0053
35	0.03313	0.04053	0.04205	0.04126	0.04881	0.0412	0.0056
50	0.03857	0.04546	0.05000	0.05487	0.05718	0.0492	0.0075
65	0.04481	0.05478	0.05605	0.06214	0.06490	0.0565	0.0078
80	0.04768	0.05968	0.06123	0.06951	0.07347	0.0623	0.0100
95	0.05265	0.06536	0.06715	0.07307	0.08016	0.0677	0.0102
110	0.05572	0.06873	0.07115	0.08089	0.08600	0.0725	0.0117
125	0.05558	0.06978	0.07149	0.08230	0.08848	0.0735	0.0127
140	0.05751	0.07159	0.07269	0.08417	0.09062	0.0753	0.0128
155	0.05674	0.07026	0.07386	0.08529	0.09189	0.0756	0.0137
185	0.06024	0.07549	0.07786	0.09041	0.09680	0.0802	0.0142
215	0.06486	0.08056	0.08201	0.09663	0.10320	0.0855	0.0150
245	0.06679	0.08340	0.08513	0.10008	0.10712	0.0885	0.0157
275	0.06902	0.08553	0.08773	-	0.11007	0.0881	0.0169
1346	0.07514	0.09311	0.09483	-	0.11970	0.0957	0.0183

Table C.2.1.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
5	11.8	1.0
20	24.4	4.0
35	30.9	4.2
50	36.9	5.6
65	42.4	5.8
80	46.7	7.5
95	50.8	7.7
110	54.4	8.8
125	55.1	9.5
140	56.5	9.6
155	56.7	10.2
185	60.1	10.6
215	64.1	11.2
245	66.4	11.8
275	66.1	12.7
1346	71.8	13.7

C.2.2 Diffusion experiment with tartrazine (1 mM) after optimization

Table C.2.2.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) in Matrigel.

	Absorbance				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	0.1400	0.1481	0.1512	0.1531	0.1597
60	0.1504	0.1537	0.1535	0.1518	0.1580
90	0.1370	0.1328	0.1305	0.1361	0.1359
120	0.1119	0.1091	0.0295	0.0320	0.1120
150	0.0919	0.0866	0.0892	0.0902	0.0908
180	0.0714	0.0689	0.0688	0.0738	0.0686
210	0.0526	0.0522	0.0532	0.0554	0.0548
240	0.0445	0.0406	0.0426	0.0443	0.0410
272	0.0340	0.0341	0.0347	0.0346	0.0319
1446	0.0689	0.0577	0.0631	0.0594	0.0563

Table C.2.2.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) in Matrigel.

	Diffused tartrazine concentration (mM)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	0.03542	0.03747	0.03826	0.03874	0.04041	0.0381	0.0018
60	0.05577	0.05763	0.05797	0.05778	0.06018	0.0579	0.0016
90	0.07140	0.07178	0.07157	0.07301	0.07458	0.0725	0.0013
120	0.08239	0.08259	0.08193	0.08494	0.08573	0.0835	0.0017
150	0.09148	0.09070	0.09106	0.09319	0.09453	0.0922	0.0016
180	0.09792	0.09717	0.09719	0.10045	0.10040	0.0986	0.0017
210	0.10220	0.10167	0.10194	0.10513	0.10559	0.1033	0.0019
240	0.10680	0.10533	0.10599	0.10933	0.10903	0.1073	0.0018
270	0.10978	0.10883	0.10938	0.11248	0.11191	0.1105	0.0016
1446	0.12291	0.11911	0.12096	0.12313	0.12212	0.1216	0.0017

Table C.2.2.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	28.5	1.4
60	43.4	1.2
90	54.4	1.0
120	62.6	1.3
150	69.1	1.2
180	74.0	1.3
210	77.5	1.4
240	80.5	1.3
270	82.9	1.2
1446	91.2	1.2

C.2.3 Verification diffusion experiment with tartrazine (1 mM)

Table C.2.3.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) in Matrigel.

	Absorbance				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	0.1666	0.1854	0.1903	0.1797	0.1977
60	0.169	0.1857	0.1982	0.1937	0.1974
90	0.18	0.1764	0.1793	0.1756	0.1740
120	0.1467	0.1464	0.1465	0.1466	0.1451
150	0.1257	0.1213	0.1196	0.1206	0.1158
180	0.0987	0.0957	0.0961	0.0974	0.0923
210	0.0800	0.0779	0.0758	0.0783	0.0747
240	0.0627	0.0602	0.0597	0.0602	0.0585
270	0.0495	0.0479	0.0466	0.0473	0.0467
1571	0.0625	0.0713	0.0823	0.0799	0.0909

Table C.2.3.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) in Matrigel.

	Diffused tartrazine concentration (mM)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	0.03275	0.03644	0.03741	0.03532	0.03886	0.03616	0.00231
60	0.04960	0.05473	0.05766	0.05574	0.05824	0.05519	0.00344
90	0.06837	0.07115	0.07343	0.07122	0.07304	0.07144	0.00200
120	0.07951	0.08259	0.08461	0.08278	0.08446	0.08279	0.00205
150	0.08980	0.09205	0.09372	0.09208	0.09296	0.09212	0.00147
180	0.09685	0.09894	0.10085	0.09937	0.09972	0.09915	0.00147
210	0.10288	0.10484	0.10631	0.10519	0.10533	0.10491	0.00126
240	0.10734	0.10902	0.11059	0.10932	0.10949	0.10915	0.00118
270	0.11091	0.11252	0.11389	0.11271	0.11292	0.11259	0.00108
1440	0.11833	0.12183	0.12548	0.12376	0.12620	0.12312	0.00317

Table C.2.3.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	27.1	1.7
60	41.4	2.6
90	53.6	1.5
120	62.1	1.5
150	69.1	1.1
180	74.4	1.1
210	78.7	0.9
240	81.9	0.9
270	84.4	0.8
1440	92.3	2.4

C.2.4 Diffusion experiment with tartrazine (1 mM) and G-block (0.5 mg/ml)

Table C.2.4.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) and G-block (0.5 mg/ml) in Matrigel.

	Absorbance				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	0.1421	0.1474	0.1211	0.1379	0.1332
60	0.1530	0.1532	0.1569	0.1549	0.1529
90	0.1343	0.1373	0.1344	0.1378	0.1420
120	0.1157	0.1133	0.1153	0.1164	0.1143
150	0.0931	0.0940	0.0976	0.0913	0.0954
180	0.0806	0.0735	0.0848	0.0753	0.0781
210	0.0596	0.0589	0.0612	0.0599	0.0598
240	0.0486	0.0476	0.0512	0.0474	0.0447
1453	0.0927	0.0914	0.0921	0.0877	0.0885

Table C.2.4.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) and G-block (0.5 mg/ml) in Matrigel.

	Diffused tartrazine concentration (mM)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	0.03595	0.03730	0.03064	0.03489	0.03370	0.03450	0.0025
60	0.05669	0.05741	0.05502	0.05664	0.05554	0.05626	0.0010
90	0.07132	0.07277	0.06918	0.07191	0.07212	0.07146	0.0014
120	0.08360	0.08407	0.08135	0.08393	0.08308	0.08320	0.0011
150	0.09252	0.09352	0.09146	0.09230	0.09276	0.09251	0.0007
180	0.10113	0.10022	0.10057	0.09981	0.10045	0.10044	0.0005
210	0.10602	0.10583	0.10532	0.10544	0.10570	0.10566	0.0003
240	0.11077	0.11042	0.11053	0.10985	0.10945	0.11021	0.0005
1453	0.12808	0.12752	0.12736	0.12604	0.12618	0.12704	0.0009

Table C.2.4.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) and G-block (0.5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	25.9	1.9
60	42.2	0.7
90	53.6	1.0
120	62.4	0.8
150	69.4	0.6
180	75.3	0.4
210	79.2	0.2
240	82.7	0.4
1453	95.3	0.7

C.2.5 Diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml)

Table C.2.5.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml) in Matrigel.

	Absorbance			
Time (min)	Well 1	Well 2	Well 3	Well 4
30	0.1552	0.1559	0.1533	0.1412
60	0.1664	0.1640	0.1607	0.1539
90	0.1376	0.1423	0.1438	0.1408
120	0.1164	0.1203	0.1193	0.1238
150	0.096	0.0991	0.0967	0.1001
180	0.0774	0.0794	0.0763	0.0843
210	0.0624	0.0652	0.0642	0.0666
240	0.0507	0.0527	0.0517	0.0518
270	0.0400	0.0409	0.0409	0.0420
1440	0.0622	0.0574	0.0638	0.0647

Table C.2.5.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml) in Matrigel.

	Diffused tartrazine concentration (mM)					
Time (min)	Well 1	Well 2	Well 3	Well 4	Mean	St. dev.
30	0.03927	0.03945	0.03879	0.03573	0.03831	0.00174
60	0.06174	0.06122	0.06006	0.05680	0.05995	0.00221483
90	0.07550	0.07648	0.07611	0.07296	0.07526	0.00158672
120	0.08755	0.08891	0.08810	0.08647	0.08776	0.00103
150	0.09711	0.09877	0.09748	0.09614	0.09737	0.00108852
180	0.10455	0.10632	0.10455	0.10480	0.10506	0.00085181
210	0.11055	0.11277	0.11114	0.11099	0.11136	0.00097
240	0.11548	0.11786	0.11610	0.11567	0.11628	0.0010857
270	0.11919	0.12154	0.11991	0.11974	0.12010	0.00101181
1440	0.12987	0.13089	0.13088	0.13080	0.13061	0.00050

Table C.2.5.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	28.7	1.3
60	45.0	1.7
90	56.4	1.2
120	65.8	0.8
150	73.0	0.8
180	78.8	0.6
210	83.5	0.7
240	87.2	0.8
270	90.1	0.8
1440	98.0	0.4

C.2.6 Diffusion experiment with tartrazine (1 mM) and G-block (0.05 mg/ml)

Table C.2.6.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) and G-block (0.05 mg/ml) in Matrigel.

	Absorbance				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	0.1514	0.1482	0.1433	0.1511	0.1539
60	0.164	0.1611	0.1562	0.1616	0.1610
90	0.1371	0.1376	0.1401	0.1394	0.1390
120	0.1116	0.1137	0.1150	0.1117	0.1110
150	0.0915	0.0925	0.0985	0.0902	0.0930
180	0.0722	0.0858	0.0764	0.0709	0.0710
210	0.0606	0.0584	0.0598	0.0606	0.0595
240	0.0486	0.0494	0.0499	0.0477	0.0469
270	0.0388	0.0387	0.0406	0.0383	0.0391
1440	0.063	0.063	0.0642	0.0583	0.0595

Table C.2.6.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) and G-block (0.05 mg/ml) in Matrigel.

	Diffused tartrazine concentration (mM)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	0.03831	0.03750	0.03626	0.03823	0.03894	0.03785	0.00102
60	0.06065	0.05951	0.05765	0.06001	0.06021	0.05961	0.00117
90	0.07459	0.07395	0.07334	0.07483	0.07501	0.07434	0.00069
120	0.08548	0.08531	0.08471	0.08546	0.08551	0.08529	0.00033
150	0.09452	0.09433	0.09509	0.09415	0.09500	0.09462	0.00041
180	0.10121	0.10434	0.10196	0.10068	0.10120	0.10188	0.00145
210	0.10741	0.10826	0.10742	0.10704	0.10727	0.10748	0.00046
240	0.11204	0.11337	0.11248	0.11145	0.11161	0.11219	0.00077
270	0.11571	0.11691	0.11644	0.11510	0.11557	0.11595	0.00072
1440	0.12674	0.12796	0.12755	0.12501	0.12568	0.12659	0.00124

Table C.2.4.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) and G-block (0.05 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	28.4	0.8
60	44.7	0.9
90	55.8	0.5
120	64.0	0.3
150	71.0	0.3
180	76.4	1.1
210	80.6	0.3
240	84.1	0.6
270	87.0	0.5
1440	94.9	0.9

C.2.7 Verification diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml)

Table C.2.7.1. Absorbance measurements of samples from diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml) in Matrigel.

	Absorbance			
Time (min)	Well 1	Well 2	Well 3	Well 4
30	0.1774	0.1829	0.1936	0.1934
60	0.1876	0.1947	0.1975	0.2030
90	0.1656	0.1690	0.1678	0.1752
120	0.1336	0.1427	0.1380	0.1473
150	0.113	0.1151	0.1086	0.1139
180	0.0875	0.0902	0.0822	0.0921
210	0.0701	0.0730	0.0653	0.0709
240	0.0540	0.0580	0.0493	0.0548
270	0.0418	0.0469	0.0377	0.0433
1571	0.0761	0.0834	0.0644	0.0746

Table C.2.7.2. Calculated total diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml) in Matrigel.

	Diffused tartrazine concentration (mM)					
Time (min)	Well 1	Well 2	Well 3	Well 4	Mean	St. dev.
30	0.03487	0.03595	0.03806	0.03802	0.03672	0.00157818
60	0.05431	0.05625	0.05785	0.05891	0.05683	0.00200448
90	0.06843	0.07033	0.07143	0.07340	0.07090	0.00207872
120	0.07841	0.08177	0.08206	0.08514	0.08185	0.00274828
150	0.08750	0.09037	0.08984	0.09305	0.09019	0.00227935
180	0.09359	0.09679	0.09533	0.09996	0.09642	0.00269952
210	0.09877	0.10228	0.10009	0.10484	0.10149	0.00266077
240	0.10249	0.10650	0.10336	0.10865	0.10525	0.00284503
270	0.10540	0.11002	0.10592	0.11177	0.10828	0.00311226
1571	0.11625	0.12181	0.11488	0.12218	0.11878	0.00375656

Table C.2.7.3. Calculated percentage diffused tartrazine and standard deviation at each time of sampling for diffusion experiment with tartrazine (1 mM) and G-block (5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	27.5	1.2
60	42.6	1.5
90	53.2	1.6
120	61.4	2.1
150	67.6	1.7
180	72.3	2.0
210	76.1	2.0
240	78.9	2.1
270	81.2	2.3
1571	89.1	2.8

C.2.8 Diffusion experiment with tartrazine (0.5 mM) and G-block (5 mg/ml)

Table C.2.8.1. Absorbance measurements of samples from diffusion experiment with tartrazine (0.5 mM) and G-block (5 mg/ml) in Matrigel.

	Absorbance		
Time (min)	Well 1	Well 2	Well 3
30	0.0830	0.1014	0.0952
90	0.1532	0.1388	0.1421
150	0.1285	0.1004	0.1210
210	0.0883	0.0800	0.0942
270	0.0725	0.0656	0.0653
1445	0.0789	0.0702	0.0721

Table C.2.8.2. Calculated total and percentage diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (0.5 mM) and G-block (5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

	Diffused tartrazine concentration (mM)						
Time (min)	Well 1	Well 2	Well 3	Mean	St. dev.	Percentage (%)	St. dev (%)
30	0.01632	0.01993	0.01871	0.01832	0.00184	27.5	2.8
90	0.03827	0.03725	0.03729	0.03760	0.00058	56.4	0.9
150	0.04848	0.04334	0.04711	0.04631	0.00266	69.5	4.0
210	0.05320	0.04920	0.05373	0.05205	0.00248	78.1	3.7
270	0.05878	0.05423	0.05731	0.05677	0.00232	85.2	3.5
1445	0.06716	0.06159	0.06507	0.06460	0.00282	96.9	4.2

C.2.9 Control diffusion experiment with tartrazine (0.5 mM)

Table C.2.9.1. Absorbance measurements of samples from diffusion experiment with tartrazine (0.5 mM) in Matrigel.

	Absorbance		
Time (min)	Well 1	Well 2	Well 3
30	0.0852	0.0786	0.0915
90	0.1327	0.1096	0.1168
150	0.1078	0.1187	0.1189
210	0.0845	0.0885	0.0890
270	0.0611	0.0539	0.0367
1445	0.0629	0.0677	0.0718

Table C.2.9.2. Calculated total and percentage diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (0.5 mM) in Matrigel. Percentage is given as the mean of the replicates.

	Diffused tartrazine concentration (mM)					Percentage	
Time (min)	Well 1	Well 2	Well 3	Mean	St. dev.	(%)	St. dev (%)
30	0.01675	0.01545	0.01799	0.01673	0.00127	25.1	1.9
90	0.03446	0.02927	0.03195	0.03189	0.00260	47.8	3.9
150	0.04261	0.04183	0.04385	0.04276	0.00102	64.1	1.5
210	0.04862	0.04756	0.04965	0.04861	0.00105	72.9	1.6
270	0.05233	0.04946	0.04812	0.04997	0.00215	75.0	3.2
1445	0.05869	0.05747	0.05863	0.05826	0.00069	87.4	1.0

C.2.10 Diffusion experiment with tartrazine (0.25 mM) and G-block (5 mg/ml)

Table C.2.10.1. Absorbance measurements of samples from diffusion experiment with tartrazine (0.25 mM) and G-block (5 mg/ml) in Matrigel.

		Absorbance	
Time (min)	Well 1	Well 2	Well 3
60	0.0580	0.0624	0.0674
150	0.0668	0.0640	0.0767
270	0.0555	0.0582	0.0638
1440	0.0474	0.0474	0.0510

Table C.2.10.2. Calculated total and percentage diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (0.25 mM) and G-block (5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

	Diffused tartrazine concentration (mM)					Percentage	
Time (min)	Well 1	Well 2	Well 3	Mean	St. dev.	(%)	St. dev (%)
60	0.01140	0.01227	0.01325	0.01231	0.00092	36.9	2.8
150	0.01883	0.01871	0.02170	0.01975	0.00169	59.2	5.1
270	0.02318	0.02386	0.02670	0.02458	0.00187	73.7	5.6
1440	0.02704	0.02746	0.03046	0.02832	0.00186	85.0	5.6

C.2.11 Control diffusion experiment with tartrazine (0.25 mM)

Table C.2.11.1. Absorbance measurements of samples from diffusion experiment with tartrazine (0.25 mM) in Matrigel.

	Absorbance		
Time (min)	Well 1	Well 2	Well 3
60	0.0642	0.0690	0.0617
150	0.0678	0.0747	0.0792
270	0.0549	0.0608	0.0630
1440	0.0482	0.0482	0.0522

Table C.2.11.2. Calculated total and percentage diffused tartrazine concentration at each sampling point for diffusion experiment with tartrazine (0.25 mM) in Matrigel. Percentage is given as the mean of the replicates.

	Diffused tartrazine concentration (mM)					Percentage	
Time (min)	Well 1	Well 2	Well 3	Mean	St. dev.	(%)	St. dev (%)
60	0.01262	0.01356	0.01213	0.01277	0.00073	38.3	2.2
150	0.01964	0.02147	0.02163	0.02091	0.00111	62.7	3.3
270	0.02377	0.02608	0.02623	0.02536	0.00138	76.1	4.1
1440	0.03324	0.03555	0.03649	0.03510	0.00167	105.3	5.0

C.2.12 Diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml)

Table C.2.12.1. Fluorescence measurements of samples from diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) in Matrigel.

	Fluorescence				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	39.7750	36.8920	41.6680	41.4490	40.5210
60	47.406	45.0390	47.3720	46.2400	43.4180
90	39.867	44.2210	44.1270	42.7210	39.5180
120	35.777	37.4580	40.0300	36.6450	38.4340
150	31.961	33.4140	30.8290	32.7080	34.4920
180	27.7680	27.3670	26.2640	27.8760	27.3570
210	23.7440	26.1160	24.1440	23.2350	26.2130
240	24.7170	25.6430	23.9990	23.4130	24.4310
270	22.7750	22.5830	23.5500	24.1640	23.7280
1440	264.343	252.518	258.189	253.353	266.172

Table C.2.12.2. Calculated total diffused IgG concentration at each sampling point for diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) in Matrigel.

	Diffused IgG concentration (ug/ml)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	2.77275	2.57178	2.90472	2.88945	2.82476	2.79269	0.13430
60	4.69109	4.42560	4.75470	4.66816	4.43909	4.59573	0.15256
90	5.81790	5.93843	6.17967	6.03456	5.68057	5.93023	0.19243
120	6.92237	7.00832	7.43213	7.10006	6.98242	7.08906	0.20217
150	7.90337	8.03203	8.18598	8.10289	8.04726	8.05430	0.10371
180	8.72509	8.77514	8.94231	8.90610	8.75210	8.82015	0.09747
210	9.41244	9.64183	9.70997	9.55420	9.62590	9.58887	0.11310
240	10.30788	10.51914	10.54141	10.37647	10.41534	10.43205	0.09789
270	11.03402	11.19962	11.34661	11.24490	11.21789	11.20861	0.11292
1440	28.66779	28.01575	28.52436	28.06413	28.94594	28.44359	0.39880

Table C.2.12.3. Calculated percentage diffused IgG and standard deviation at each time of sampling for diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	8.4	0.4
60	13.8	0.5
90	17.8	0.6
120	21.3	0.6
150	24.2	0.3
180	26.5	0.3
210	28.8	0.3
240	31.3	0.3
270	33.7	0.3
1440	85.4	1.2

C.2.13 Diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) and G-block (5 mg/ml)

Table C.2.13.1. Fluorescence measurements of samples from diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) and G-block (5 mg/ml) in Matrigel.

	Fluorescence				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	37.8140	41.9400	39.9820	41.0250	43.3370
60	49.751	47.6530	47.7980	48.5610	47.1460
90	45.076	45.5530	43.5120	45.2170	41.9960
120	40.314	38.9280	38.3870	40.5260	37.0880
152	38.101	36.9340	35.9440	37.3280	33.9950
180	31.0490	29.3990	29.5530	28.6490	27.4550
210	27.7300	27.1200	26.0170	25.6130	23.6620
240	27.5500	27.0170	25.7290	23.6470	23.2410
270	25.8030	24.1600	24.2790	24.5060	23.1150
1440	299.415	292.097	273.687	278.588	273.269

Table C.2.13.2. Calculated total diffused IgG concentration at each sampling point for diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) and G-block (5 mg/ml) in Matrigel.

	Diffused IgG concentration (ug/ml)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	2.63605	2.92368	2.78718	2.85989	3.02106	2.84557	0.14527
60	4.78621	4.78377	4.72564	4.81518	4.79712	4.78159	0.03364
90	6.19441	6.29835	6.09288	6.27468	6.08141	6.18835	0.10018
120	7.43359	7.42429	7.25224	7.52373	7.20306	7.36738	0.13447
150	8.68448	8.64214	8.41993	8.71335	8.28016	8.54801	0.18906
180	9.52091	9.40422	9.22725	9.40941	9.00916	9.31419	0.20031
210	10.37177	10.27006	10.01084	10.19634	9.70171	10.11014	0.26369
240	11.32576	11.20816	10.89760	10.95204	10.49711	10.97613	0.32105
270	12.16425	11.95069	11.69331	11.83615	11.29840	11.78856	0.32366
1440	32.13737	31.47094	29.92604	30.40262	29.54256	30.69590	1.08252

Table C.2.13.3. Calculated percentage diffused IgG and standard deviation at each time of sampling for diffusion experiment with Alexa Fluor® 488 IgG (0.25 mg/ml) and G-block (5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	8.5	0.4
60	14.4	0.1
90	18.6	0.3
120	22.1	0.4
150	25.7	0.6
180	28.0	0.6
210	30.4	0.8
240	33.0	1.0
270	35.4	1.0
1440	92.2	3.3

C.2.14 Diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml) and G-block (5 mg/ml)

Table C.2.14.1. Fluorescence measurements of samples from diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml) and G-block (5 mg/ml) in Matrigel.

	Fluorescence				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	8.176	7.655	8.706	7.888	8.012
60	9.804	8.469	9.338	9.009	9.033
90	8.343	8.770	8.407	8.350	8.441
120	7.887	7.447	7.280	7.239	7.048
150	7.228	6.625	6.847	6.156	6.072
180	6.523	5.805	6.135	5.663	5.625
210	5.402	5.228	5.556	5.220	4.774
240	5.546	4.887	4.826	4.540	4.770
270	4.986	4.705	4.510	4.237	4.188
1440	58.557	52.701	57.369	50.814	51.312

Table C.2.14.2. Calculated total diffused IgG concentration at each sampling point for diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml) and G-block (5 mg/ml) in Matrigel.

	Diffused IgG concentration (ug/ml)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	0.56996	0.53364	0.60690	0.54988	0.55852	0.56378	0.02751
60	0.96842	0.85720	0.95441	0.90297	0.90896	0.91839	0.04436
90	1.20830	1.17337	1.21499	1.17104	1.18254	1.19005	0.02032
120	1.46731	1.38683	1.42946	1.38463	1.37965	1.40958	0.03796
150	1.69628	1.58910	1.65302	1.56146	1.55727	1.61142	0.06095
180	1.89907	1.76285	1.84204	1.74166	1.73775	1.79667	0.07104
210	2.04828	1.92496	2.01552	1.90816	1.87449	1.95428	0.07406
240	2.24661	2.08342	2.15829	2.04270	2.04061	2.11433	0.08796
270	2.40088	2.24107	2.30447	2.17983	2.16630	2.25851	0.09667
1440	6.30915	5.75091	6.14652	5.57444	5.59734	5.87567	0.33356

Table C.2.14.3. Calculated percentage diffused IgG and standard deviation at each time of sampling for diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml) and G-block (5 mg/ml) in Matrigel. Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	8.5	0.4
60	13.8	0.7
90	17.9	0.3
120	21.2	0.6
150	24.2	0.9
180	27.0	1.1
210	29.3	1.1
240	31.7	1.3
270	33.9	1.5
1440	88.2	5.0

C.2.15 Control diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml)

Table C.2.15.1. Fluorescence measurements of samples from diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml).

	Fluorescence				
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5
30	6.657	6.111	5.387	6.331	6.446
60	7.876	8.235	7.847	7.871	7.860
90	7.428	7.798	7.934	7.481	7.484
120	6.668	6.600	6.828	6.451	6.703
150	6.289	6.113	6.194	6.034	4.883
180	5.253	5.735	5.402	5.648	4.443
210	5.133	4.769	5.094	4.396	4.887
240	4.154	4.265	4.226	4.325	4.716
270	4.046	3.859	4.104	4.055	3.810
1440	48.898	47.855	46.503	47.714	44.772

Table C.2.15.2. Calculated total diffused IgG concentration at each sampling point for diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml).

	Diffused IgG concentration (ug/ml)						
Time (min)	Well 1	Well 2	Well 3	Well 4	Well 5	Mean	St. dev.
30	0.46407	0.42600	0.37553	0.44134	0.44936	0.43126	0.03405
60	0.78108	0.78707	0.73479	0.76936	0.77261	0.76898	0.02035
90	1.02437	1.04364	1.01436	1.01652	1.02036	1.02385	0.01170
120	1.23029	1.23193	1.21381	1.20548	1.22677	1.22166	0.01151
150	1.43629	1.42803	1.40760	1.40126	1.33354	1.40134	0.04053
180	1.58327	1.61475	1.56829	1.58467	1.47306	1.56481	0.05399
210	1.75801	1.74730	1.73511	1.69425	1.65888	1.71871	0.04126
240	1.86867	1.87840	1.85215	1.84253	1.81729	1.85181	0.02382
270	2.00593	1.99875	1.99094	1.97446	1.91851	1.97772	0.03510
1440	5.27363	5.20026	5.08967	5.15931	4.90681	5.12594	0.13945

Table C.2.15.3. Calculated percentage diffused IgG and standard deviation at each time of sampling for diffusion experiment with Alexa Fluor® 488 IgG (0.05 mg/ml). Percentage is given as the mean of the replicates.

Time (min)	Percentage (%)	St. dev. (%)
30	6.5	0.5
60	11.5	0.3
90	15.4	0.2
120	18.3	0.2
150	21.0	0.6
180	23.5	0.8
210	25.8	0.6
240	27.8	0.4
270	29.7	0.5
1440	77.0	2.1

Appendix D: Statistical analyses of diffusion experiments

D.1 Verification of tartrazine diffusion curve

Table D.1 demonstrates p-values from the statistical analysis comparing the diffused concentration of tartrazine from Matrigel in control experiment 1 and control experiment 2. The confidence interval was 95 % and a T-test was used.

Table D.1. Statistical analysis comparing diffused tartrazine concentration in control 1 and control 2 at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	Control 1 ≠ Control 2	0.19	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
60	Control 1 ≠ Control 2	0.16	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
90	Control 1 ≠ Control 2	0.37	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
120	Control 1 ≠ Control 2	0.56	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
150	Control 1 ≠ Control 2	0.94	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
180	Control 1 ≠ Control 2	0.62	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
210	Control 1 ≠ Control 2	0.16	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
240	Control 1 ≠ Control 2	0.09	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
270	Control 1 ≠ Control 2	0.05	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.
≈ 1440 (24 hours)	Control 1 ≠ Control 2	0.39	Diffusion of tartrazine from Matrigel in control 1 was not significantly different from diffusion of tartrazine from Matrigel in control 2. H1 is rejected.

D.2 Comparison of tartrazine diffusion in diffusion experiment with G-block (0.5 mg/ml) and control, both with tartrazine (1 mM)

Table D.2 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a diffusion experiment with G-block (0.5 mg/ml) and control experiment, both with tartrazine (1 mM). The confidence interval was 95 % and a T-test was used.

Table D.2. Statistical analysis comparing diffused tartrazine concentration in diffusion experiment with G-block (0.5 mg/ml) and control at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block < Control	0.038	Diffusion of tartrazine in control was significantly higher than diffusion of tartrazine from Matrigel with G-block. H1 is accepted.
60	G-block > Control	0.087	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
90	G-block > Control	0.274	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
120	G-block > Control	0.744	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
150	G-block > Control	0.703	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
180	G-block > Control	0.068	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
210	G-block > Control	0.051	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected..
240	G-block > Control	0.018	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
≈ 1440 (24 hours)	G-block > Control	0.001	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.

D.3 Comparison of tartrazine diffusion in diffusion experiment with G-block (5 mg/ml) and control, both with tartrazine (1 mM)

Table D.3 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a diffusion experiment with G-block (5 mg/ml) and control experiment, both with tartrazine (1 mM). The confidence interval was 95 % and a T-test was used.

Table D.3. Statistical analysis comparing diffused tartrazine concentration in diffusion experiment with G-block (5 mg/ml) and control at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block > Control	0.840	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
60	G-block > Control	0.172	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
90	G-block > Control	0.031	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
120	G-block > Control	0.002	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
150	G-block > Control	0.001	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
180	G-block > Control	0.000	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
210	G-block > Control	0.000	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
240	G-block > Control	0.000	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
270	G-block > Control	0.000	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
≈ 1440 (24 hours)	G-block > Control	0.000	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.

D.4 Comparison of tartrazine diffusion in diffusion experiment with G-block (0.05 mg/ml) and control, both with tartrazine (1 mM)

Table D.4 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a diffusion experiment with G-block (0.05 mg/ml) and control experiment, both with tartrazine (1 mM). The confidence interval was 95 % and a T-test was used.

Table D.4. Statistical analysis comparing diffused tartrazine concentration in diffusion experiment with G-block (0.05 mg/ml) and control at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block > Control	0.828	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
60	G-block > Control	0.087	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
90	G-block > Control	0.032	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
120	G-block > Control	0.083	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
150	G-block > Control	0.023	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
180	G-block > Control	0.011	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
210	G-block > Control	0.009	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
240	G-block > Control	0.003	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
270	G-block > Control	0.000	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
≈ 1440 (24 hours)	G-block > Control	0.001	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.

D.5 Comparison of tartrazine diffusion in diffusion experiment with G-block (5 mg/ml) and G-block (0.05 mg/ml), both with tartrazine (1 mM)

Table D.5 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a diffusion experiment with G-block (5 mg/ml) and G-block (0.05 mg/ml), both with tartrazine (1 mM). The confidence interval was 95 % and a T-test was used.

Table D.5. Statistical analysis comparing diffused tartrazine concentration in diffusion experiment with G-block (5 mg/ml) and G-block (0.05 mg/ml) at various sampling points.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.660	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is rejected.
60	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.790	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is rejected.
90	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.341	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is rejected.
120	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.010	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.
150	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.010	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.
180	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.0046	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.
210	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.002	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.
240	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.001	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.

270	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.001	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.
≈ 1440 (24 hours)	G-block (5 mg/ml) > G-block (0.05 mg/ml)	0.001	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was significantly higher than diffusion of tartrazine from Matrigel with G-block (0.05 mg/ml). H1 is accepted.

D.6 Comparison of tartrazine diffusion in verification diffusion experiment with G-block (5 mg/ml) and control, both with tartrazine (1 mM)

Table D.6 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a verification diffusion experiment with G-block (5 mg/ml) and control, both with tartrazine (1 mM). The confidence interval was 95 % and a T-test was used.

Table D.6. Statistical analysis comparing diffused tartrazine concentration in verification diffusion experiment with G-block (5 mg/ml) and control at various sampling points.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block (5 mg/ml) verification > control	0.278	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
60	G-block (5 mg/ml) verification > control	0.431	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
90	G-block (5 mg/ml) verification > control	0.247	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
120	G-block (5 mg/ml) verification > control	0.337	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
150	G-block (5 mg/ml) verification > control	0.198	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.

180	G-block (5 mg/ml) verification > control	0.211	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
210	G-block (5 mg/ml) verification > control	0.302	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
240	G-block (5 mg/ml) verification > control	0.266	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
270	G-block (5 mg/ml) verification > control	0.270	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.
≈ 1440 (24 hours)	G-block (5 mg/ml) verification > control	0.228	Diffusion of tartrazine from Matrigel with G-block (5 mg/ml) was not significantly different from diffusion of tartrazine from Matrigel in control. H1 is rejected.

D.7 Comparison of tartrazine diffusion in diffusion experiment with G-block (5 mg/ml) and control, both with tartrazine (0.5 mM)

Table D.7 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a diffusion experiment with G-block (5 mg/ml) and control experiment, both with tartrazine (0.5 mM). The confidence interval was 95 % and a T-test was used.

Table D.7. Statistical analysis comparing diffused tartrazine concentration in diffusion experiment with G-block (5 mg/ml) and control at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block > Control	0.285	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
90	G-block > Control	0.065	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
150	G-block > Control	0.120	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
210	G-block > Control	0.114	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
270	G-block > Control	0.020	Diffusion of tartrazine from Matrigel with G-block was significantly higher than diffusion of tartrazine in control. H1 is accepted.
≈ 1440 (24 hours)	G-block > Control	0.063	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.

D.8 Comparison of tartrazine diffusion in diffusion experiment with G-block (5 mg/ml) and control, both with tartrazine (0.25 mM)

Table D.8 demonstrates p-values from the statistical analysis comparing the diffused tartrazine concentration from Matrigel in a diffusion experiment with G-block (5 mg/ml) and control experiment, both with tartrazine (0.25 mM). The confidence interval was 95 % and a T-test was used.

Table D.8. Statistical analysis comparing diffused tartrazine concentration in diffusion experiment with G-block (5 mg/ml) and control at various sampling times.

Sampling time (min)	**Hypothesis (H1)**	**p-value**	**Conclusion**
60	G-block > Control	0.531	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
150	G-block > Control	0.393	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
270	G-block > Control	0.594	Diffusion of tartrazine from Matrigel with G-block was not significantly higher than diffusion of tartrazine in control. H1 is rejected.
≈ 1440 (24 hours)	G-block < Control	0.009	Diffusion of tartrazine in control was significantly higher than diffusion of tartrazine from Matrigel with G-block. H1 is accepted.

D.9 Comparison of Alexa Fluor® 488 IgG diffusion in diffusion experiment with G-block (5 mg/ml) and control, both with IgG (0.25 mg/ml)

Table D.9 demonstrates p-values from the statistical analysis comparing the diffused Alexa Fluor® 488 IgG concentration from Matrigel in a diffusion experiment with G-block (5 mg/ml) and control experiment, both with IgG (0.25 mg/ml). The confidence interval was 95 % and a T-test was used.

Table D.9. Statistical analysis comparing diffused IgG concentration in diffusion experiment with G-block (5 mg/ml) and control at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block > Control	0.567	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was not significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is rejected.
60	G-block > Control	0.056	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was not significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is rejected.
90	G-block > Control	0.038	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
120	G-block > Control	0.037	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
150	G-block > Control	0.002	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
180	G-block > Control	0.003	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
210	G-block > Control	0.010	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
240	G-block > Control	0.015	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
270	G-block > Control	0.013	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
≈ 1440 (24 hours)	G-block > Control	0.007	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.

D.10 Comparison of Alexa Fluor® 488 IgG diffusion in diffusion experiment with G-block (5 mg/ml) and control, both with IgG (0.05 mg/ml)

Table D.10 demonstrates p-values from the statistical analysis comparing the diffused Alexa Fluor® 488 IgG concentration from Matrigel in a diffusion experiment with G-block (5 mg/ml) and control experiment, both with IgG (0.05 mg/ml). The confidence interval was 95 % and a T-test was used.

Table D.10. Statistical analysis comparing diffused IgG concentration in diffusion experiment with G-block (5 mg/ml) and control at various sampling times.

Sampling time (min)	Hypothesis (H1)	p-value	Conclusion
30	G-block > Control	0.000	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
60	G-block > Control	0.001	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
90	G-block > Control	0.000	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
120	G-block > Control	0.000	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
150	G-block > Control	0.000	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
180	G-block > Control	0.001	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
210	G-block > Control	0.001	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
240	G-block > Control	0.001	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
270	G-block > Control	0.002	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.
≈ 1440 (24 hours)	G-block > Control	0.006	Diffusion of Alexa Fluor® 488 IgG from Matrigel with G-block was significantly higher than diffusion of Alexa Fluor® 488 IgG in control. H1 is accepted.

Appendix E: Example of rheological raw data

The raw data presented in this appendix are results from the rheological measurements of Matrigel from the temperature ramp (section 3.2.2). The data is given as an example of rheological data obtained in this thesis. Rheological data for all experiments described in section 3.2, Rheological characterization of Matrigel are enclosed in a zip-file.

E.1 Oscillation strain control

Table E.1.1. Rheological data obtained from oscillation strain control measurements of Matrigel from temperature ramp. Frequency = 1 Hz. Strain = $5*10^{-3}$.

Time (min)	Temperature (ºC)	Phase angle (δ)	G' (Pa)	G'' (Pa)
0.3	37.9	7.8	3.57E+01	4.88E+00
0.7	37.2	6.2	5.39E+01	5.85E+00
0.8	37.1	8.4	5.90E+01	8.74E+00
1.2	37	6.3	6.66E+01	7.37E+00
1.7	37.2	4.7	7.53E+01	6.26E+00
2.1	37.1	5.5	7.90E+01	7.64E+00
2.4	37	5.1	8.14E+01	7.28E+00
2.7	37	4.9	8.23E+01	6.99E+00
3.2	37.1	5.1	8.37E+01	7.51E+00
3.3	37	4.3	8.46E+01	6.38E+00
3.6	37.1	4.3	8.53E+01	6.46E+00
3.9	37	5	8.70E+01	7.53E+00
4.1	37	4.7	8.62E+01	7.06E+00
4.4	37	4.6	8.67E+01	6.94E+00
4.5	36.9	4.2	8.71E+01	6.47E+00
4.7	37	4.4	8.81E+01	6.75E+00
5.0	37.1	4.3	8.82E+01	6.62E+00
5.1	37.1	4.4	8.82E+01	6.76E+00
5.3	37	4.2	8.81E+01	6.40E+00
5.5	37	4.2	8.87E+01	6.58E+00
5.6	37	4.4	8.87E+01	6.82E+00
5.8	37	4.2	8.89E+01	6.58E+00
5.9	37	4.2	8.86E+01	6.44E+00
6.1	36.9	4	8.83E+01	6.15E+00
6.2	37.1	4.2	8.87E+01	6.47E+00
6.4	37	4.3	8.90E+01	6.76E+00
6.7	36.9	4	8.95E+01	6.34E+00
6.8	36.9	4.5	8.91E+01	7.03E+00
7.0	37	4.4	8.88E+01	6.76E+00
7.2	37	4.1	8.90E+01	6.42E+00
7.3	37	4.1	8.93E+01	6.42E+00
7.5	37	4.4	8.99E+01	6.95E+00
7.6	37	4.3	8.97E+01	6.72E+00

7.8	37	4.2	8.94E+01	6.55E+00
7.9	37	4.3	8.96E+01	6.72E+00
8.2	37	4.5	9.02E+01	7.12E+00
8.4	37	4.2	8.93E+01	6.62E+00
8.6	37	4.2	8.97E+01	6.63E+00
8.7	37	4.2	8.98E+01	6.66E+00
8.9	37	4.1	8.99E+01	6.49E+00
9.0	37	4.1	9.00E+01	6.49E+00
9.2	37.1	4.2	9.03E+01	6.64E+00
9.3	37	3.8	8.99E+01	5.93E+00
9.5	36.9	4.2	9.02E+01	6.55E+00
9.7	37	4.1	9.00E+01	6.39E+00
9.8	37	4	9.04E+01	6.28E+00
10.0	37	4.2	9.07E+01	6.69E+00
10.1	37	4.1	9.03E+01	6.44E+00
10.3	37	4.1	9.03E+01	6.53E+00
10.4	37	4.1	9.00E+01	6.41E+00
10.7	37	4.3	9.06E+01	6.79E+00
10.9	37	3.9	9.04E+01	6.20E+00
11.0	37	4.5	9.02E+01	7.02E+00
11.2	37	3.6	9.08E+01	5.70E+00
11.4	37	4	9.02E+01	6.29E+00
11.5	37	4.2	9.07E+01	6.70E+00
11.7	37	4.1	9.06E+01	6.47E+00
11.8	37	4.3	9.15E+01	6.86E+00
12.0	37	4.2	9.05E+01	6.68E+00
12.1	37	4.2	9.03E+01	6.66E+00
12.3	37	4	9.09E+01	6.29E+00
12.5	37	4.1	9.09E+01	6.44E+00
12.6	37	4	9.07E+01	6.40E+00
12.8	37	4.2	9.08E+01	6.60E+00
12.9	37.1	3.9	9.04E+01	6.13E+00
13.1	37	3.8	9.05E+01	6.02E+00
13.2	37	4.1	9.06E+01	6.43E+00
13.4	37	4.1	9.05E+01	6.56E+00
13.6	37	4	9.01E+01	6.28E+00
13.7	37	4.5	9.05E+01	7.06E+00
13.9	37	4	9.11E+01	6.43E+00
14.0	37	4	9.12E+01	6.31E+00
14.2	37	4.1	9.09E+01	6.51E+00
14.3	37	3.8	9.08E+01	6.01E+00
14.5	37	3.9	9.06E+01	6.25E+00
14.6	36.9	4.1	9.07E+01	6.50E+00
14.8	37	3.7	9.09E+01	5.95E+00
15.0	37	4	9.00E+01	6.35E+00
15.1	36.9	3.9	9.09E+01	6.19E+00
15.3	37	4	9.06E+01	6.40E+00
15.4	37	4	9.11E+01	6.45E+00
15.6	37	4	9.15E+01	6.33E+00
15.7	37	3.6	9.08E+01	5.75E+00
15.9	37	3.8	9.05E+01	6.02E+00
16.1	37	3.7	9.09E+01	5.85E+00
16.2	37	3.5	9.07E+01	5.52E+00

16.4	37	4.2	9.10E+01	6.74E+00
16.5	37	4.4	9.07E+01	6.90E+00
16.7	37	4	9.12E+01	6.35E+00
16.8	37	4.1	9.06E+01	6.48E+00
17.0	37	4.1	9.11E+01	6.51E+00
17.1	36.9	3.5	9.10E+01	5.61E+00
17.3	37	4	9.10E+01	6.36E+00
17.5	37	3.8	9.07E+01	6.01E+00
17.6	37	3.9	9.09E+01	6.27E+00
17.8	37	3.9	9.05E+01	6.12E+00
17.9	37	3.8	9.08E+01	6.02E+00
18.1	36.9	4.1	9.10E+01	6.48E+00
18.2	37	3.9	9.07E+01	6.21E+00
18.4	37	4	9.04E+01	6.31E+00
18.5	37	3.4	9.11E+01	5.39E+00
18.7	37	4.2	9.11E+01	6.74E+00
18.9	36.9	3.8	9.07E+01	6.10E+00
19.0	37	4	9.08E+01	6.36E+00
19.2	37	3.8	9.04E+01	5.96E+00
19.3	37	3.9	9.03E+01	6.09E+00
19.5	37	3.5	9.04E+01	5.52E+00
19.6	37	3.6	9.07E+01	5.67E+00
19.8	36.9	3.9	9.04E+01	6.16E+00
20.0	37	3.7	9.06E+01	5.89E+00
20.1	37	3.8	9.03E+01	5.94E+00
20.3	37	3.8	9.10E+01	6.06E+00
20.4	37.1	3.6	9.05E+01	5.65E+00
20.6	37	3.6	9.04E+01	5.71E+00
20.7	37	3.7	9.07E+01	5.91E+00
20.9	37	4	9.04E+01	6.35E+00

E.2 Frequency sweep

Table E.2.1. Rheological data obtained from frequency sweep of Matrigel from temperature ramp. Frequency region: $5*10^{-3}$-10 Hz. Temperature = 37 ºC. Strain = $5*10^{-3}$.

Frequency (Hz)	Phase angle (δ)	G' (Pa)	G'' (Pa)
5.00E-03	17.9	6.11E+01	1.98E+01
7.00E-03	14	6.28E+01	1.57E+01
1.00E-02	10.9	6.58E+01	1.27E+01
1.50E-02	8.7	6.79E+01	1.04E+01
2.00E-02	7.1	6.91E+01	8.62E+00
3.00E-02	6	6.95E+01	7.35E+00
4.00E-02	5.3	7.05E+01	6.59E+00
5.00E-02	5.5	7.07E+01	6.77E+00
6.00E-02	5.1	7.10E+01	6.36E+00
7.00E-02	4.8	7.18E+01	6.09E+00
8.00E-02	5	7.17E+01	6.28E+00
9.00E-02	4.9	7.25E+01	6.21E+00
1.00E-01	4.8	7.23E+01	6.01E+00
1.50E-01	4.9	7.41E+01	6.39E+00
2.00E-01	4.9	7.49E+01	6.39E+00
3.01E-01	4.6	7.69E+01	6.20E+00
4.00E-01	4.6	7.75E+01	6.19E+00
5.00E-01	4.2	7.86E+01	5.81E+00
6.01E-01	4.2	7.95E+01	5.87E+00
7.00E-01	3.9	7.95E+01	5.45E+00
8.00E-01	3.9	8.03E+01	5.43E+00
9.00E-01	3.7	8.01E+01	5.16E+00
1.00E+00	3.8	8.01E+01	5.33E+00
1.50E+00	3.2	8.12E+01	4.50E+00
2.00E+00	3	8.18E+01	4.24E+00
3.00E+00	3	8.22E+01	4.33E+00
4.00E+00	3.1	8.29E+01	4.43E+00
5.00E+00	3.5	8.32E+01	5.07E+00
6.00E+00	3.2	8.36E+01	4.72E+00
7.00E+00	3.3	8.39E+01	4.82E+00
8.00E+00	3.3	8.39E+01	4.83E+00
9.00E+00	3.3	8.37E+01	4.76E+00
1.00E+01	3.2	8.34E+01	4.69E+00

E.3 Strain sweep

Table E.3.1 Rheological data obtained from strain sweep of Matrigel from temperature ramp. Strain region: $1*10^{-4}$-1. Frequency = 1 Hz. Temperature = 37 ºC**.**

Strain	Phase angle (δ)	G' (Pa)	G'' (Pa)
1.02E-04	2.9	7.43E+01	3.77E+00
7.33E-05	3.1	1.24E+02	6.68E+00
1.45E-04	4.8	9.53E+01	7.93E+00
2.16E-04	7.6	7.15E+01	9.53E+00
2.71E-04	7.6	7.27E+01	9.66E+00
2.41E-04	0.2	7.66E+01	3.19E-01
2.99E-04	1.6	8.79E+01	2.48E+00
3.93E-04	1.5	7.51E+01	2.03E+00
4.50E-04	2.6	8.19E+01	3.71E+00
5.03E-04	3.2	7.96E+01	4.47E+00
6.26E-04	5.5	8.11E+01	7.76E+00
8.11E-04	4.2	7.57E+01	5.62E+00
9.82E-04	2.6	7.69E+01	3.46E+00
1.15E-03	2.5	7.96E+01	3.54E+00
1.39E-03	2.8	7.79E+01	3.79E+00
1.66E-03	2.9	7.79E+01	3.96E+00
2.02E-03	3.6	7.78E+01	4.88E+00
2.47E-03	3	7.75E+01	4.11E+00
2.99E-03	3.9	7.73E+01	5.31E+00
3.57E-03	3.2	7.73E+01	4.28E+00
4.28E-03	3.4	7.78E+01	4.57E+00
5.16E-03	3.5	7.73E+01	4.68E+00
6.22E-03	3.2	7.68E+01	4.32E+00
7.50E-03	3.4	7.70E+01	4.63E+00
9.05E-03	3.3	7.70E+01	4.47E+00
1.09E-02	3	7.68E+01	3.99E+00
1.31E-02	3.3	7.71E+01	4.40E+00
1.59E-02	3.4	7.68E+01	4.52E+00
1.93E-02	3.4	7.67E+01	4.61E+00
2.31E-02	3.4	7.68E+01	4.61E+00
2.79E-02	3.5	7.66E+01	4.66E+00
3.37E-02	3.4	7.67E+01	4.60E+00
4.06E-02	3.3	7.69E+01	4.47E+00
4.90E-02	3.3	7.68E+01	4.43E+00
5.91E-02	3.3	7.69E+01	4.45E+00
7.13E-02	3.3	7.70E+01	4.48E+00
8.60E-02	3.3	7.72E+01	4.47E+00
1.05E-01	3.3	7.75E+01	4.41E+00
1.25E-01	3.3	7.79E+01	4.44E+00
1.52E-01	3.2	7.87E+01	4.42E+00
1.84E-01	3.2	7.99E+01	4.42E+00
2.21E-01	3.1	8.18E+01	4.40E+00
2.66E-01	3	8.46E+01	4.44E+00
3.20E-01	2.9	8.91E+01	4.46E+00
3.84E-01	2.7	9.62E+01	4.53E+00

4.59E-01	2.5	1.08E+02	4.64E+00
5.46E-01	2.2	1.26E+02	4.91E+00
6.46E-01	2	1.58E+02	5.43E+00
7.61E-01	2.2	2.14E+02	8.19E+00
9.08E-01	2.5	3.08E+02	1.35E+01