Photochromic properties of a spiropyran photoswitch molecule in skin tissue models

Master thesis in Engineering Physics

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Abstract

In chemotherapy, one problem is that the substances used do not always show high specific affinity to tumor cells. It is then of great interest to find other possible ways of treating cancer patients, using a more local treatment. An already existing local cancer treatment is photo dynamic therapy, where a substance is applied locally to cancerous tissue and made toxic by irradiating the treated area. The substances used produce cytotoxic singlet oxygen when irradiated, leading to the death of cancer cells. Another possibility, for a similar local cancer treatment, could be to use spiropyran molecules. Spiropyrans are photoswitch molecules and it was discovered that a certain spiropyran molecule, in combination with UV radiation, caused human embryonic kidney (HEK) 293T cells (kidney tumor cells) to die. Thus, if the spiropyran could be locally applied to cancer cells followed by activation of its cytotoxic effect by UV irradiation a selected area could be treated.

Skin is the tissue that is most easily treated with spiropyrans and reached by UV radiation. Therefore, as a first step towards a local cancer treatment, it was suggested to start studying the distribution of spiropyrans in skin tissue. Skin samples from mouse and pig were used as a model for human skin and treated with the spiropyran and scanned in a confocal microscope in order to detect how the spiropyran distributes in the skin. These experiments showed that the spiropyran could penetrate skin tissue, but the efficiency depended on the application method and the type of skin tissue used.

In another experiment, Franz cells were used to investigate the permeability of skin for the spiropyran. The Franz cell experiments show that the spiropyran did not penetrate the skin tissue used, under the specific conditions of the experiment in this project. In general, pigskin and human skin seemed more resilient to the spiropyran than mouse skin but further experiments with Franz cells are needed to evaluate if the spiropyran can penetrate human skin under certain conditions.
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Introduction
Photoswitch molecules are molecules that can exist in two different forms and switching between the forms is done by using radiation. The photoswitch used in this project, a spiropyran molecule, seems to have one form that is cytotoxic and one that is not. The cytotoxic form of the molecule is obtained by irradiating the non-cytotoxic form with UV radiation.\textsuperscript{1}

The cancer type that has the highest chance of being reached by UV radiation is skin cancer. Other cancer types, that might be deeper inside the body, would have more tissue in the way that absorbs UV radiation, resulting in the UV not reaching the cancer cells.

As a first step towards a potential future skin cancer treatment, experiments were made to see if the spiropyran would at all penetrate skin tissue, and if so, what the distribution of the spiropyran in the different parts of the skin would look like. Since experiments like these had not been made previously, there was no protocol to follow. Therefore, a lot of time was spent developing appropriate experiments that would answer the questions asked above.

The skin tissue used in this project was for the most part dead, so the cytotoxic effects of the spiropyran in skin were not investigated. This is left as future work.

Spiropyran molecules
Spiropyrans are a family of photoswitch molecules, meaning radiation is used to cause a reversible transformation between two (or sometimes more) different forms of the molecule, with different absorption spectra.

Spiropyrans were first synthesized and studied in the beginning of the 1950\textquotesingle s, by a research group in Israel, lead by Hirschberg and Fisher.\textsuperscript{2} As mentioned above, spiropyrans are photoswitch molecules. UV light and visible light are used to switch between the two forms of the spiropyran. The two forms have different properties.

6-nitro BIPS
The particular spiropyran molecule used in the present project was a 6-nitro BIPS.

The two molecular forms of the 6-nitro BIPS spiropyran are the closed, spiro (SP) form and the open, merocyanin (MC) form. They differ in three dimensional structure, absorption spectra, fluorescence emission and polarity.\textsuperscript{1}

The following information about the properties of the spiropyran refers to the 6-nitro BIPS.

Properties in aqueous solution
The spiropyran does not have a photocromic (switchable by using radiation) behavior in solid state. In this state it has an appearance of a yellow powder. When dissolved in water, conversion of SP into MC is achieved by irradiating the solution with UV light. Using an UV light source of 254 nm, the spiropyran will end up in a stationary state with a ratio between SP and MC of 40/60. The time constant is 4.7 min.

The reverse reaction, that is the conversion of MC into SP, is obtained by exposing the solution to visible light, with $\lambda > 465$ nm. This will convert almost all of the spiropyran into SP.
Figure 1. The 6 nitro BIPS, and the reversible reaction between its two states: SP and MC.

SP has absorption peaks at 270 nm and 351 nm, while MC has absorption peaks at 356 nm and 510 nm. The emission maximum of MC is found at 641 nm.

Figure 2. Absorption spectrum of a solution containing the spiropyran.
Before exposing the solution to UV, there is very little absorption at 510 nm (green line) which is one of the absorption maxima of MC. After only 30 s of UV exposure (pink line), there is an increase in the absorption at 510 nm, indicating the presence of MC.
Continuing to expose the solution to UV light, the absorption peak at 510 nm grows, showing the conversion of SP into MC. The reverse reaction, conversion of MC into SP is achieved by exposing the
solution to visible light. After a total of 4 min visible light exposure (dotted line), there is almost no MC left in the solution.

Heat, not only UV, can also convert SP into MC, although the reaction is much faster using UV. In an aqueous solution, left in the dark at 23 °C, the spiropyran reaches an equilibrium state where the ratio between SP and MC is 50/50, after approximately 6 hours.

**Photoswitching - ring opening reaction**

The SP consists of two parts, one pyran part and one heterocyclic part. These two parts are connected via a carbon atom called the spiro carbon, in such a way that they lie in planes perpendicular to each other. The electrons of the pyran part and the heterocyclic part are in so called π orbitals, which are certain electronic molecular states. The structure of SP, together with the shape of the electronic orbitals, makes it impossible for the electronic orbitals of the two parts to interact, resulting in the molecules absorption spectrum essentially being the sum of the absorption spectra of the two individual parts.

The electrons of the spiro carbon are not in π orbitals, but instead in so called sp³- hybrid orbitals (Figure 3a.), which are electronic states that are combinations of atomic electronic s- and p-states.

When exposing SP to heat and/or UV radiation, the electrons of the molecule are excited to higher energy states and the bond between the oxygen atom in the pyran part of the molecule and the spiro carbon atom to break (Figure 4). The electrons of the spiro carbon go into other states: sp²- hybrid orbitals (Figure 3b.), which are other combinations of s- and p-atomic electronic states. This allows for the two parts of the molecule to rotate relative each other and results in the molecule assuming a more planar shape. In this form, the electrons of the molecule can interact across the entire molecule, giving the molecule a different set of properties than in its SP form, for example having a different absorption spectrum and being fluorescent³⁻⁸. This reaction is often referred to as “the ring opening reaction”, since the carbon ring containing the oxygen atom of the pyran part of the molecule opens. Drawings of the conversion of SP into MC, the reverse reaction and the fluorescence emission of MC are shown in the following two pages.

![Figure 3. The sp³-hybrid orbitals of carbon, a., before bonding. Four of carbons six electrons will form these states, when carbon is surrounded by atoms eager to participate in reactions. The formation of sp²-hybrid orbitals (blue lobes) are shown in b., together with one p orbital (green lobes), also before bonding.](image-url)
Figure 4. Photoswitching of the spiropyran. Red arrow indicates which bond that breaks in the conversion of SP to MC.

SP to MC conversion

Figure 5. Drawing above shows the conversion of SP to MC. UV radiation excites the ground state SP into an excited molecular electronic state, SP*, shown in a., and from there it is deexcited to the MC ground state, shown in b.
**MC to SP conversion**

![Diagram showing energy transitions between MC and SP forms, with a peak at VIS (λ=488 nm).]

**Figure 6.** Visible light causes the MC form of the spiropyran to convert back to its SP form.

**MC fluorescence**

![Diagram showing energy transitions for MC fluorescence, with peaks at VIS (λ=488 nm) and another peak centered around 641 nm.]

**Figure 7.** If the MC form binds to something (for example an organelle or molecule in a cell or to some structure in some kind of tissue) and cannot convert back to SP when exposed to visible light, shown in a., it is forced into the excited MC state: MC*. When deexcited from there back to the MC ground state, it emits fluorescent light (b.).

**Intermediate states**

The SP form does not immediately convert to the final MC form when exposed to UV, but it also forms intermediate states. When the bond between the spiro carbon and the oxygen in the pyran breaks, the molecule starts to become more planar, but it does not become completely planar until it has reached...
its final MC state. The intermediate states will all have higher molecular energies compared to the final
MC state, which is why this MC a more stable state than the intermediate states.6

**Polarity**
The polarity of the spiropyran differs between SP and MC. SP is non-polar while MC is polar. As a
consequence, the behavior of the spiropyran is affected by the polarity of the solvent.
In general, a more polar solvent will favor the ground state MC and the conversion of MC to SP is slower
and requires more energy, meaning a blue shifted MC absorption band, relative to using a non-polar
solvent.3

**Photoswitch molecules**
There are many biological applications in which photoswitch molecules, not only spiropyran
photoswitches, are used. A few of these include activating and deactivating biologically active
molecules, such as proteins9-12, and imaging cells.13-15
Because of the two state nature of photoswitch molecules, it has also been suggested that they may be
useful in biological systems for mimicking logic gates.16

**Activation of biomolecules**
There are mainly two ways of controlling the activation of biomolecules, when using photo-sensitive
molecules. The first, most common, involves using photo caging groups. Photo caging groups are light-
sensitive molecules that are bound to biologically active molecules, in such a way that the biomolecule
becomes deactivated (for example shielding the active site of a protein). Irradiation of the biomolecule-
photo cage causes the photo cage to detach from the biomolecule, making the biomolecule active and
ready to react. However, this process will be irreversible.
To be able to turn the activation of a biomolecule in to a reversible process, photoswitch molecules can
be used instead of photo cages. As an example of this, researchers created a molecular valve, using a
channel protein and a spiropyran molecule.10 The spiropyran was bound to the channel protein and then
embedded in a membrane. The spiropyran was attached to the channel protein so that one of the
spiropyran’s two forms would cause the valve to be closed and the other form would cause it to be open.
Switching between the two forms of the spiropyran, using radiation, was then equivalent to switching
between a closed and an open molecular valve.

**Imaging of live cells**
Spiropyran photoswitches embedded in hydrophobic cavities in polymeric nanoparticles might serve as
useful devices to image the insides of cells. Researchers successfully managed to incorporate these
spiropyran-nanoparticle complexes into HEK293 cells, via vesicles.13 Once inside the cells, they were able
to cycle between the spiropyran two forms, using radiation. The nanoparticles were needed to shield
the spiropyran from the cellular environment as it affects its photochemical properties. Since one of the
spiropyran’s two forms is fluorescent while the other is not, switching the spiropyran’s into its fluorescent
form makes imaging possible.
Skin tissue
Skin tissue is a very complex and dynamic type of tissue. Although living skin was not used in this project, it was still important to learn the basics of skin structure and function to be able to draw the right conclusions about the behavior of the spiropyran in skin.

Epidermis
Epidermis is the outermost layer of the skin and in turn consists of four different layers. Beginning with the deepest layer, these are: the basal layer (stratum basale), the spinous layer (stratum spinosum), the granular layer (stratum granulosum) and the cornified layer (stratum corneum). Sometimes, in thicker parts of the skin (found in humans in the palm of hands and the sole of feet), a fifth layer is included between the granular layer and the cornified layer, called the clear/translucent layer (stratum lucidum). The total thickness of epidermis varies depending on location, and is between 0.05 mm (eyelids) and 1.5 mm (palms).

In the basal layer the stem cells of the skin are found. They are attached to a basement membrane below, and to more differentiated skin cells above, through different cell adhesions. While most of the skin stem cells will differentiate into keratinocytes, others will become melanocytes. Melanocytes produce melanin, which is a substance used to protect the cell nuclei of the keratinocytes from harmful radiation.

Keratinocytes make a journey upwards in the skin, through the different cell layers, to finally die before being cast off the skin surface. The most superficial skin layer, stratum corneum, consists of mostly dead skin cells. The purpose of the keratinocytes is to produce the protein keratin, which functions as a rigid grid of protection against the external environment.

Langerhans cells are cells part of the immune system. They are found squeezed in between the keratinocytes in epidermis and have dendritic tails shooting out between them. They use these tails to detect antigens in order to present these to other cells in the immune system.

Dermis
Dermis consists of two layers: the papillary layer and the reticular layer, where the papillary layer is the more superficial layer, in contact with epidermis through a basement membrane. The thickness of dermis, like epidermis, depends on location, and is between 0.3 mm (eyelids) and 3 mm (back). Epidermis is not parallel to dermis (Figure 8), but instead makes projections into dermis, called epidermal ridges. The space between these projections is filled with parts of dermis, called dermal papillae.

The portion of the papillary layer closest to epidermis is mostly made up of loose connective tissue; mainly collagen fibers and elastic fibers. The fibers are thinner in this layer than the fibers in deeper parts of dermis.
The papillary layer also contains hair follicles, nerve endings, sweat glands, and blood vessels that are in contact with (without entering) epidermis.
The reticular layer lies beneath the papillary layer, and consists of collagen fibers and stronger elastic fibers, than the papillary layer. It is also thicker than the papillary layer, although its thickness varies in different parts of the body. The collagen and elastic fibers in the reticular layer are oriented in a structured way, making up regular lines of tension in the skin. Cutting (when operating) parallel to these lines will result in wound healing with the least scarring.

**Hypodermis**

Beneath the reticular layer there is a layer of mainly fat cells which varies in thickness, called hypodermis. The thickness of this layer varies greatly between individuals and is between 2 mm and 10 mm, being thicker in fat people. The purpose of the layer is to function as insulation, and is also thicker in individuals living in cold climates. 17-18

![Image of a skin cross-section.](image)

**Skin cancer**

Skin cancer is usually divided into two main groups: Non Melanoma Skin Carcinoma (NMSC) and Melanoma Skin Carcinoma (MSC). Most commonly among NMSC is Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC).

BCC is due to damaged DNA of stem cells in the basal layer of epidermis, causing them to develop into cancer cells. BCC is not likely to spread to others parts of the body and is therefore rarely responsible for any deaths. It accounts for 80–85 % of all NMSC. 19

Cancer cells classified as SCC mimic the keratinocytes in the spinous layer of epidermis, and can grow...
either upwards towards the skin surface, or downwards into dermis. If SCC penetrates dermis, it can come in contact with blood vessels and spread to other parts in the body.\textsuperscript{20} Melanoma is the most lethal type of skin cancer but fortunately also rarer than NMSC. It is due to damaged melanocytes.\textsuperscript{20}

**Human skin models**

The type of skin that has proved to be most like human skin when it comes to the permeation of different substances, is pig skin. Researchers tested the permeability of different human skin models for a variety of different substances with different polarity.\textsuperscript{21} They used pig skin, rat skin and two types of artificial human skin; Graftskin and HRE (Human reconstructed epidermis). The fluxes of the substances through the different skin types were measured with the help of Fanz cells. The fluxes through the pig skin were in the same order of magnitude as the fluxes through the human skin, regardless of which substance used. The rat skin showed, for some of the substances, a substantially higher flux than human skin, as did the artificial human skin types.

In this project pig skin and mouse skin was used. In the Franz cell experiment human skin was used. The reason for using mouse skin in some of the experiments, although pig skin is a much more suitable model for human skin, was that it was easier to get a hold of fresh mouse skin than fresh pig skin. It was important to use skin that had not been dead for too long (not more than 2-3 hours) as this increased the chance of the skin staying intact during the experiments and would therefore be easier to study in a microscope.

**Specific aims**

The aim of the project was to investigate, and to develop appropriate experiments to test if the spiropyran would at all penetrate skin tissue. If so, it was also examined how deep in to the skin the penetration would be and if the spiropyran would prefer certain skin layers more than others.

To try to answer the questions above practically, skin samples from pig and mice were incubated with the spiropyran and scanned with a laser of a confocal microscope in order to be able to detect the spiropyran. UV radiation was used to excite the spiropyran once inside the skin samples, to convert the spiropyran to its fluorescent, and therefore detectable, open MC form.

Another experiment that was made was to test the permeability of skin tissue for the spiropyran. To examine this, a so called Franz cell experiment was carried out, where human breast skin was used.
Methods

Preparation of spiropyran
The spiropyran used in this project was synthesized by Shimming Li at Chalmers.

Spectroscopy
The spiropyran was dissolved in water. To obtain the desired concentration an absorption spectrum of the spiropyran water solution was measured. In aqueous solution, at 298 nm, the two forms (SP and MC) absorb radiation equally well. \( \lambda = 298 \text{ nm} \) is referred to as the spiropyran isosbestic point. Together with Beer-Lambert’s law this information was used to calculate the total concentration of the spiropyran in the solution.

Expressed in terms of absorbance, Beer-Lambert’s law can be expressed as:

\[ A_\lambda = \varepsilon cl \]

where

- \( c \) = concentration of substance dissolved in liquid sample \( \text{[M]} \)
- \( l \) = path length traveled by the radiation through the sample \( \text{[cm]} \)
- \( \varepsilon \) = molar absorption coefficient \( \text{[M}^{-1}\text{cm}^{-1}] \)

As stated above, in an aqueous solution, at 298 nm, the two forms of the spiropyran absorb equally well. This means that the absorption measured at this wavelength can be used to calculate the total concentration of the spiropyran in the solution, when also knowing the molar absorption coefficient at this wavelength, \( \varepsilon_{\lambda=298} \), which is equal to 7369 \( \text{M}^{-1}\text{cm}^{-1} \).

The absorbance of a spiropyran water solution was measured at 298 nm using a photospectrometer. The concentration was calculated using the above equation, \( A_{\lambda=298} = \varepsilon cl \), where \( l \) was equal to 1 cm. With \( A_{\lambda=298} \), \( l \) and \( \varepsilon_{\lambda=298} \) known, \( c \) could be calculated. The desired concentration was then obtained by diluting the solution.

The spiropyran was also dissolved in DMSO, in order to treat skin samples with this solution as well. Additionally the spiropyran DMSO solution was mixed with a skin cream, to apply to other skin samples. However, for most of the skin cream-mixes containing the spiropyran, the spiropyran was first dissolved in water instead of DMSO, and then mixed with the skin cream.

The skin cream used was called Essex skin cream, bought at a pharmacy and is used as a base in many other skin creams. The cream was a water-based cream.

Skin samples

Pig skin
Skin samples were obtained from a pig’s ear. A set of experiments were made to see if the spiropyran would be able to penetrate the skin when applied in different ways. The spiropyran was dissolved in
water, with a concentration of 30 µM spiropyran. The spiropyran was also dissolved in DMSO, where the intention again was to prepare a solution of 30 µM, but unfortunately it was quite difficult to calculate the concentration when using DMSO as a solvent: the behavior of the spiropyran is different in DMSO than in water, because of the different polarity of the solvents. The isosbestic point at λ = 298 nm used to calculate the concentration of the spiropyran in water, disappears when the spiropyran is dissolved in DMSO.

The spiropyran was applied to four different skin samples, in four ways. Each experiment had a control sample associated with it, where the control sample was treated as the samples exposed to the spiropyran in all ways except being in contact with the spiropyran.

Table 1. The spiropyran was applied to different pig skin samples, in four different ways:

<table>
<thead>
<tr>
<th>Applying the spiropyran to pig skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. placed in a SP water solution</td>
</tr>
<tr>
<td>2. SP water drop placed on skin surface</td>
</tr>
<tr>
<td>3. SP DMSO drop placed on skin surface</td>
</tr>
<tr>
<td>4. SP DMSO cream placed on skin surface</td>
</tr>
</tbody>
</table>

Skin cryosectioning
To be able to study the penetration and the distribution of the spiropyran in the skin in more detail, cryosections of the skin samples were cut. Skin cryosections are slices cut perpendicular to the skin surface, making it possible to see the different layers of the skin.

It could be expected that there is more of the molecule closer to the skin surface, or edges, of the skin samples incubated with the spiropyran. It would then be easier to detect the molecule there, since it is more easily reached by the UV radiation used to convert the SP form into the MC fluorescent detectable form. This could give a false image of how deep inside the skin the spiropyran reaches. However, studying skin cryosections solves this problem: no matter how deep the spiropyran penetrates, it will have an equal chance of being reached by the UV radiation, since each layer of the skin cryosection is
Before cutting cryosections, the skin samples were placed in a glue-like substance called trissuetek, in order to fixate the samples, and then frozen with dry ice. The samples were cut using a cryostat, a compartment equipped with a knife, held at -23 °C. The cryosections of the samples were collected immediately after cutting, by placing them on cover glasses.

**Pig skin cryosections**
Skin samples used for cutting pig skin cryosections were obtained from the skin of a pig’s abdomen. These were incubated with the spiropyran, in three different ways, before cryosectioning.

**Table 2.** Spiropyran applied to different pig skin samples, in three different ways:

<table>
<thead>
<tr>
<th>Applying the spiropyran to pig skin (cryosections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. SP water solution</td>
</tr>
<tr>
<td>2. SP water drop</td>
</tr>
<tr>
<td>3. SP cream</td>
</tr>
</tbody>
</table>

The skin samples were also prepared before cutting by being placed in a sugar solution for approximately 24 hours, after they had been treated with the spiropyran. This was done in order to increase the stability of the skin samples and make them less affected by the tissuetek used to fixate the skin samples before cutting.

**Mouse skin cryosections**
Mouse skin was also used, the reason being that it was easier to get a hold of fresh mouse skin than fresh pig’s skin. There is a higher chance of the skin layers staying intact if using fresh skin when cutting cryosections, and it is therefore easier to study the distribution of the spiropyran in the different skin layers.

Two sets of mouse skin cryosection experiments were performed. In both sets, skin pieces from the back of a mouse were used; this skin was first shaved and then cut into smaller skin pieces. These pieces were
treated with the spiropyran in three different ways, the same three different ways as the pig skin used for cryosectioning, each case having a control.

In the first set of mouse skin cryosection experiments, the skin samples had only been treated with the spiropyran before being cut into cryosections. In the second set of mouse skin cryosection experiments, the skin was, like the pig skin used for cryosectioning, prepared by being placed in a sugar solution for approximately 24 hours before cutting.

The skin samples from both pig and mouse were in contact with the spiropyran between 17 and 24 hours, while left in the dark in a fridge, at 4 °C.

In all the experiments, before applying the spiropyran to the skin pig and mouse skin, almost all of the spiropyran was in its SP form, which was achieved by exposing the spiropyran solutions used to visible light. The reason for this was to try to have as much of the spiropyran in its SP form as possible once inside the skin, so that there hopefully would be more of the spiropyran to convert to its fluorescent MC form.

**Human skin**

In the Franz cell experiments human breast skin was used, which had been donated to Sahlgrenska University Hospital by women who had had breast reduction surgery.

**Confocal microscopy**

Using a confocal microscope it is possible to generate 3D images from a stack of 2D images. In a confocal microscope only small spots (points) of the sample are illuminated at a time, in comparison with wide field microscopy where a larger area of the sample is illuminated. Additionally, a pinhole aperture is placed at the back focal plane of the objective in the microscope, allowing only light originating from the illuminated spot at the focal point to pass through the pinhole and be detected. By refocusing the objective of the microscope, spots at other depths in the sample can be illuminated in the same point like manner. Scanning a 2D surface of many small spots, or points, results in a 2D image. Obtaining several of these 2D images of slices at different depths of the sample and combining these slices results in a 3D image.

**Figure 9.** Schematic drawing of the confocal microscope. Drawing is from the compendium “Light microscopy” (2007), by Kjell Carlsson at Applied Physics Department, KTH.
Skin samples from the ear of a pig were prepared with the spiropyran and scanned in a confocal microscope, parallel to the skin surface. The laser used to scan the samples was a 488 nm laser, and the filter used for detection was a long pass filter with $\lambda > 505$ nm. The objective used was a 10X objective. A halogen lamp with an UV filter ($\lambda$ centered around 254 nm) was used to convert the spiropyran in the skin samples from its SP form to its MC form.

In order to detect the spiropyran in the skin samples, some chosen spots of each sample were scanned before and after UV exposure. Before UV exposure a set of 10 images were collected, with a 5 second interval between every image. Then the same spot was exposed to UV radiation for 10 seconds. Another set of images were collected immediately after, to see if there was any change in fluorescence intensity that would indicate the presence of the spiropyran. This procedure was then repeated once. The skin was viewed from the skin surface some distance into the skin. The fluorescence intensity in the images were measured in ImageJ, and plotted versus the number of images (frames).

When scanning the cryosections in the confocal microscope, again a few spots were scanned both before and after UV exposure. To sample more data, a set of 30 images were collected with a time interval of 2 seconds before UV exposure and then also after. This was repeated twice. The images were analyzed in the same way as the images of the (non-cryosections) pig skin.

For the mouse skin cryosection, other measurements of the fluorescence in the images were also made, so called fluorescence intensity profiles, to try to get some sort of indication of how deep into the skin the spiropyran had managed to penetrate, and/or the distribution of the spiropyran in the skin. A more detailed explanation of the fluorescence intensity profile-analysis is presented under “Results”.

**Franz cell**

To measure how well the spiropyran penetrates skin, a set of experiments using Franz cells were carried out.

Franz cells are often used to measure how well a substance penetrates some type of tissue. The experimental setup is quite simple: a sample of the tissue used is placed between two compartments, the upper, donor compartment and the lower, receptor compartment (Figure 10). The substance is applied to the tissue through the donor compartment, while the receptor compartment is filled with some type of fluid, usually water, called the receptor fluid. A part of the receptor fluid is collected at some time after having applied the substance to the tissue, and an absorption spectrum of the receptor fluid is measured to see if there is any of the substance that has managed to penetrate the tissue all the way through and ended up in the receptor fluid.
Figure 11. Drawing of the Franz cell.

Human breast skin was used in the Franz cell experiment, and four creams of different spiropyran concentrations were prepared by mixing a spiropyran water solution (concentration: 472 µM) with a skin-cream. One cream that didn’t contain any spiropyran was also prepared and used as a control.

Table 3. The five different cream-mixes that were applied to skin in the Franz cell experiments.

<table>
<thead>
<tr>
<th>Cream</th>
<th>Mix</th>
<th>Time cream-mix was applied to skin 2011-11-03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream 1</td>
<td>60 µL spiropyran solution + 25g cream</td>
<td>10.30</td>
</tr>
<tr>
<td>Cream 2</td>
<td>50 µL spiropyran solution + 10 µL water + 25 g cream</td>
<td>10.40</td>
</tr>
<tr>
<td>Cream 3</td>
<td>40 µL spiropyran solution + 20 µL water + 25 g cream</td>
<td>10.50</td>
</tr>
<tr>
<td>Cream 4</td>
<td>30 µL spiropyran solution + 30 µL water + 25 g cream</td>
<td>11.05</td>
</tr>
<tr>
<td>Cream 5</td>
<td>Control: 0 µL spiropyran solution + 60 µL water + 25 cream</td>
<td>11.14</td>
</tr>
</tbody>
</table>
The cream-mixes were applied to the skin surface of five different skin samples, and the skin samples were placed between the two different compartments of the Franz cell. The skin surface was in contact with the upper compartment, while the other side of the skin was in contact with the bottom compartment, which also contained a receptor fluid. The Franz cells were placed in a water bath of 35 °C, which is approximately the temperature at the skin surface. Five samples of the receptor fluid were collected, from each one of the France cells, at three different times.

**Table 4.** Time at which the receptor fluids were sampled, from each Franz cell.

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<tr>
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<tbody>
<tr>
<td>Cream 1</td>
<td>12.55</td>
<td>15.51</td>
<td>08.50</td>
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<tr>
<td>Cream 2</td>
<td>12.57</td>
<td>15.53</td>
<td>08.51</td>
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<tr>
<td>Cream 3</td>
<td>12.59</td>
<td>15.55</td>
<td>08.52</td>
</tr>
<tr>
<td>Cream 4</td>
<td>13.02</td>
<td>15.57</td>
<td>08.53</td>
</tr>
<tr>
<td>Cream 5</td>
<td>13.04</td>
<td>16.00</td>
<td>08.54</td>
</tr>
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Absorption spectrum measurements of 1 ml of the receptor fluid mixed with 0.5 ml water were made to see if there would be any absorption peaks indicating the presence of SP and/or MC. That is, to see if the spiropyran had penetrated the skin.
Results

Spiropyran distribution in pig skin

Skin samples from a pig’s ear (Figure 12) were incubated with the spiropyran in the three different ways (Figure 13). A few of the microscope images of the pig skin samples are shown below (Figure 14), where five different spots were imaged before and after UV, for each skin sample. A bright field image, an image before UV exposure and an image after UV exposure, of one spot from each skin sample is shown. An image of a control that hadn’t been exposed to the spiropyran is also shown (Figure 14, bottom row) for comparison.

![Figure 12. Skin samples from a pig’s ear](image)

![Figure 13. Pig skin samples treated with the spiropyran in three different ways.](image)

![Figure 14. Microscope images of pig skin samples incubated with the spiropyran. Notice the increase in fluorescence intensity after UV exposure in the skin sample that had been placed in a spiropyran water solution (top row). Red arrows in the images correspond to 200 µm.](image)
The images were analyzed in ImageJ, where the fluorescence intensity in each image was measured, before and after each set of UV radiation dose. The graph below (Figure 15) shows the variation in fluorescence intensity in the images. Colors indicate in which way the skin sample was treated with the spiropyran. For each skin sample, five different spots were exposed to two sets of 10 second UV doses, at times (frames) 10 and 20, and a set of 10 images was collected before and after each UV dose. The values of the fluorescence intensity are plotted versus the number of frames, where a set of 10 images corresponds to 55 seconds.

The fluorescence intensity values plotted are the mean values from the five different spots of each skin sample. The pig skin sample placed in a spiropyran water solution (blue line) indicates the presence of the spiropyran in the skin by the measured increase in fluorescence intensity after each dose of UV radiation, followed by the gradual decrease in fluorescence intensity in the sets of images taken after each UV dose (using 488 nm laser = visible light). The presence of the spiropyran could not be established in the skin samples incubated with the spiropyran in the other two ways (by placing a spiropyran water drop on the skin surface (red line) and placing a spiropyran DMSO drop on the skin surface (green line)).

**Figure 15.** Fluorescence intensity variation in images taken of pig skin samples incubated with the spiropyran. The standard error of the mean (SEM) is shown as error bars.
Another pig skin sample was exposed to a spiropyran cream. Different microscope setting were used (a slightly higher detector gain for the fluorescence detection channel) when viewing this skin sample, therefore these results are shown separately.

The spiropyran was dissolved in DMSO and mixed with a skin cream which was applied to the surface of the pig skin sample (Figure 16). The skin sample was viewed in a microscope and exposed to UV radiation. Images below (figure 17) show the bright field image of a spot of the skin sample under study, the spot before UV exposure and the spot after UV exposure. The bottom row shows the images of a spot of a control skin sample, only treated with a skin cream mixed with DMSO.

**Figure 16.** Pig skin samples treated with a SP cream.

**Figure 17.** Microscope images of a pig skin sample treated with a spiropyran cream. Red arrows in the images correspond to 200 µm.

Figure 18 below shows the fluorescence intensity increase and decrease in the images of the skin sample treated with the SP cream, and the control skin sample associated with it (indicated by different colors), before and after two sets of UV radiation. The skin sample was exposed to 10 seconds of UV radiation at times (frames) 10 and 20. The values of the fluorescence intensity are mean values from five different spots of each skin sample.

The variation of the fluorescence intensity before and after UV for the skin sample treated with the spiropyran cream (blue line) shows the typical behavior of the spiropyran.

**Figure 18.** Fluorescence intensity variation in images taken of a pig skin sample incubated with the spiropyran, when exposed to UV radiation.
Spiropyran distribution in pig skin cryosections

In order to study the distribution of the spiropyran in skin samples more carefully, skin cryosections were cut using pig skin taken from the abdomen of a pig (Figure 19). Skin samples were incubated in two different ways (Figure 20). Microscope images of spots of a few of the pig skin cryosections are shown in Figure 21. Images of a spot of a cryosection from a control skin sample is shown in the bottom row for comparison.

**Figure 19.** Pig skin samples, from the abdomen of a pig.

**Figure 20.** Pig skin samples, used for cryosectioning, treated with the spiropyran in two different ways.

**Figure 21.** Images of pig skin cryosections exposed to the spiropyran. In the tile images the spot of the cryosection under study is shown, indicated by the red square. Also shown: the bright field image of the spot, and the spot before and after UV exposure. The skin surface is determined by noting the auto fluorescence from the first skin layer (stratum corneum) and is in the fluorescence images seen as a bright green line. Red arrows in the images correspond to 200 µm.

The graph below (Figure 22) shows the fluorescence intensity in the pig skin cryosections, before and after three sets of 10 seconds UV doses. 30 images were collected before and after each UV dose. A set of 30 images corresponds to 89 seconds. The UV doses were applied to the skin at times (frames) 30, 60
and 90. For each skin sample, the values of the fluorescence intensity are mean values from five different spots plotted versus the number of that image (frame). Colors indicate type of skin sample. Although the fluorescence intensity in the images of the control skin sample is the highest (black line) it does not change when exposed to UV and visible light as if the spiropyran would be present in the skin sample. The fluorescence measured is instead autofluorescence. In the SP water solution skin sample (blue line), the typical fluorescence intensity change, when exposed to UV radiation, is seen due to the presence of the spiropyran. This could unfortunately not be shown for the SP water drop with the microscope settings used in this experiment.

![Mean fluorescence intensity decay and SEM, pig skin cryosections](image)

**Figure 22.** The variation in fluorescence intensity in the images taken of pig skin cryosections incubated with the spiropyran, when exposed to UV radiation.
Another pig skin sample was incubated with a spiropyran cream, but different microscope setting were used (a slightly higher detector gain for the fluorescence detection channel) when viewing the cryosections cut from this pig skin sample (Figure 23), therefore the results are shown separately. Microscope images below (Figure 24) show the fluorescence and bright field images of a few of the pig skin cryosections.

Figure 23. Pig skin samples treated with a SP cream. The skin samples were later cut into cryosections.

Figure 24. Microscope images of pig skin cryosections. Shown are the tile images (row furthest to the left) where the spot under study is indicated by a red square, the bright field images (middle row) and the fluorescence images (before and after UV exposure) of the same spot. The fluorescence images indicate the skin surface the by the bright green fluorescing line. Red arrows in the images correspond to 200 µm.

Figure 25 below shows the mean values of the fluorescence intensity in the images taken of the pig skin cryosections cut from the skin sample treated with the SP, and its associated control skin sample. Five different spots of each skin sample was studied, before and after three sets of UV radiation doses. The skin cryosections were exposed to UV radiation at frames 30, 60 and 90. The cryosections cut from the skin sample treated with the SP cream (blue) shows the typical change in fluorescence intensity of the spiropyran when alternately exposed to UV and visible light.

Figure 25. Fluorescence intensity variation in the images taken of pig skin cryosections incubated with a spiropyran cream, and its associated control skin sample.
Spiropyran distribution in mouse skin cryosections; first set of experiments

Mouse skin from the back of a mouse (Figure 26) was also used in order to study the spiropyran in skin tissue. Mouse skin samples were treated with the spiropyran in three different ways (Figure 27) and cut into cryosections. Figure 28 shows a few of the microscope images of these cryosections. The images shown of the cryosections are bright field images and images before and after UV exposure. For comparison, the bottom row shows the images of a cryosection cut from a control skin sample.

![Figure 26. Skin from a mouse’s back, cut into smaller skin samples.](image)

<table>
<thead>
<tr>
<th>SP water solution</th>
<th>SP water drop</th>
<th>SP cream</th>
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![Figure 27. The skin samples from the mouse’s back treated with the spiropyran in three different ways, before cryosectioning.](image)

![Figure 28. Microscope images of skin cryosections cut from mouse skin samples treated with the spiropyran. Red arrows in the images correspond to 200 µm. For the SP water solution cryosection and the SP water drop cryosection the red arrow is pointing away from the side of the cryosections which is the skin surface, and for the SP cream cryosection it is pointing towards the skin surface.](image)

The results from the analysis of the microscope images taken of the mouse skin cryosections treated with the spiropyran is shown in the graph below (Figure 29). The graph shows the mean values of the fluorescence intensity measured from the images taken of the mouse skin cryosections. 30 sets of
images were collected before and after three sets of 10 seconds UV doses, for five different spots of each skin sample. The UV doses were applied at times (frames) 30, 60 and 90. The variation of the fluorescence intensity from the SP water solution skin sample and the SP cream, when alternately exposed to UV and visible light, indicate the presence of the spiropyran. This is however not seen in the cryosections cut from the skin sample exposed to a SP water drop.

-Spiropyran distribution in mouse skin cryosections; second set of experiments-

The purpose of doing a second set of mouse skin cryosection-experiments was for the most part simply to repeat the first one, in order to see if the results would be similar. However, one modification of the experiments was made: adding a step where the mouse skin samples were placed in a sugar solution after spiropyran incubation, and left in that solution for approximately 24 hours. This was to increase the stability of the skin samples, resulting in it being easier to cut cryosections.

In these experiments, again, mouse skin from the back of a mouse was used (Figure 30). However this time, the skin cryosections cut from the mouse skin sample incubated with the spiropyran water drop was viewed in the microscope with slightly different settings (higher detector gain for the fluorescence channel), than the cryosections cut from the mouse skin samples incubated with the spiropyran water solution and the spiropyran cream (Figure 31). Therefore the images and results of the spiropyran water drop skin cryosections are shown separately.

The bright field images of the cryosections from the spiropyran water solution skin sample and spiropyran cream skin sample, and the images before and after UV are shown in Figure 32. The images of a control skin sample (bottom row) are included for comparison.

Figure 29. Variation in fluorescence intensity in images taken of mouse skin cryosections cut from skin samples treated with the spiropyran.
The graph below (Figure 33) shows the mean values of the fluorescence intensity measured from the images taken of mouse skin cryosections, from five different spots, for each skin sample. The UV radiation doses were applied at times (frames) 30, 60 and 90. Both the variation in fluorescence intensity in the skin samples treated with the spiropyran water solution (blue line) and the SP cream (red line) indicate the presence of the spiropyran.

**Figure 30.** Skin from a mouse’s back cut into smaller skin samples. Also shown is the shaving of the skin. After the skin samples had been treated with the spiropyran, they were placed in a sugar solution for approximately 24 hours before cryosectioning.

**Figure 31.** Mouse skin samples treated with the spiropyran in two different ways.

**Figure 32.** Microscope images of mouse skin cryosections, from skin treated with the spiropyran in different ways.

**Figure 33.** Variation of the fluorescence intensity when mouse skin cryosections were exposed to UV radiation.
As stated previously, different microscope setting were used (a slightly higher detector gain for the fluorescence detection channel) when viewing the cryosections cut from the mouse skin sample treated with the spiropyran water drop, therefore these results are shown separately. The reason using different setting was because of the difficulties in detecting the spiropyran in the skin sample treated in this way in the first set of mouse skin cryosection-experiments. The skin samples (Figure 34) together with the microscope images of the skin cryosections (Figure 35) are shown below.

**Figure 34.** Mouse skin sample treated with a SP water drop.

**Figure 35.** Microscope images of mouse skin cryosections, cut from a skin sample treated with a SP water drop. Images shown are the bright field image of the cryosection, and the images of the cryosection before and after UV exposure. Bottom row shows the images of a cryosection cut from a control skin sample, for comparison.

The graph below show the mean values of the fluorescence cryosection cut from the SP water drop skin sample (blue), and its associated control (red), from five different spots of each skin sample. The change in fluorescence intensity in the SP water drop skin cryosection, when alternately exposed to UV and visible light, show the presence of the spiropyran. The cryosections were exposed to three UV radiation doses at times (frames) 30, 60 and 90.

**Figure 36.** Variation in fluorescence intensity when the skin cryosections cut from mouse skin samples were exposed to UV radiation.
Fluorescence intensity profile in mouse skin cryosections; first set of experiments

In order to try to get an understanding of how deep the spiropyran would penetrate the skin samples, another analysis of the mouse skin cryosections were made. The variation in fluorescence intensity was measured along lines, indicated by the red arrows in the images, which correspond to 300 µm each. Shown below are a few of the images analyzed (Figure 37). For each case of treating the skin samples with the spiropyran (SP water solution, SP water drop, SP cream), five fluorescence intensity profiles were measured on five different spots.

![Figure 37](image)

The result of the measured fluorescence intensity along the lines shown in the images in Figure 37 is shown in Figure 38. The values plotted are mean values from five measurements of five different spots for each case of treating the skin with the spiropyran. From Figure 37 it is clear that there is more of the spiropyran closer to the edges of the skin samples, i.e. to where it was applied, as could be expected.

![Figure 38](image)
Fluorescence intensity profile in mouse skin cryosections; second set of experiments

The measurements and analysis of the fluorescence intensity profiles of the second set of mouse skin cryosection-experiment (prepared with a sugar solution before cutting and after being treated with the spiropyran) were made in the same as for the first set of mouse skin cryosection-experiments. Some of the analyzed images are shown in Figure 39, where the fluorescence intensity was measured along the red lines (arrows). Each red line corresponds to 300 µm. For each case of treating the skin samples with the spiropyran (water solution, drop or cream) five measurements were made from five different images, except for the spiropyran water solution case, where five measurements were made from each side of the cryosections.

<table>
<thead>
<tr>
<th>SP water solution</th>
<th>SP water drop</th>
<th>SP cream</th>
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**Figure 39.** Measurements of the fluorescence intensity profiles of mouse skin cryosections.

The analysis of the measured fluorescence along the lines in the images of the cryosections (Figure 39) is shown in the graph below (Figure 40).

**Figure 40.** Graph shows the mean values of the variation of fluorescence intensity through skin cryosections. The mean values were smoothed in MATLAB before plotting.
**Franz cell**

In the Franz cell experiment human breast skin was used. Images below (Figure 41) show the breast skin samples. Before using the samples they were filleted, in this case meaning the bottom fat layer of the skin was cut off, which is shown in the image above furthest to the right.

![Figure 41. Human breast skin.](image)

In the Franz cell experiment, five different skin samples were used, where each skin sample was placed in a Franz cell. The skin surface of each skin sample was exposed to a SP cream (Figure 42, image furthest to the left). The Franz cells were then placed in a water bath (Figure 42, middle image) which was held at 35 °C (Figure 42, image furthest to the right). Five different cream-mixes were used, four containing different amounts of SP and one control-cream, without any SP. The creams were applied to the skin samples between 10.30 and 11.14, 2011-11-03.

![Figure 42. Franz cell experimental set-up.](image)

Three different absorption spectrum measurements were made at different times, for each of the Franz cells.
Figure 43 shows the first absorption spectrum measurement of the Franz cell receptor fluid. The receptor fluid from each Franz cell was collected between 12.55 and 13.04, 2011-11-03. No absorption peaks that would indicate the presence of the spiropyran were seen. If there would have been any, or enough of the spiropyran in the receptor fluid to detect, there would have been absorption peaks at 351 nm and/or 510 nm.

The second absorption spectrum measurement of the receptor fluid of each Franz cell is shown in Figure 44. The receptor fluids were collected between 15.51 and 16.00, 2011-11-03. Again, there were no absorption peaks that pointed towards there being any (or at least not enough to detect) spiropyran in the receptor fluids.

The third measurement of the absorption spectrum of the receptor fluid of each Franz cell is shown below in Figure 45. The receptor fluids were collected between 08.50 and 08.54, 2011-11-04, that is, the day after the creams were applied to the skin. As in the first and second absorption spectrum measurements, no spiropyran could be detected in this measurement either.
After the last absorption spectrum measurement of the receptor fluid was carried out, the part of the skin samples that had been in contact with the SP cream were cut out and each placed in a plastic tube containing DMSO. The purpose was to try to draw the spiropyran out of the skin, assuming that it was somehow stuck in the skin. The skin was left in the DMSO for approximately 2 hours. An absorption spectrum of the DMSO was measured after that.

Figure 47 below shows the absorption spectrum of the DMSO that had been in contact with the skin pieces from the Franz cell experiment exposed to the SP cream. There are no absorption peaks indicating the presence of the spiropyran in the DMSO, for any of the different skin samples. For spiropyran dissolved in DMSO, there should be some absorption around 366 nm and/or 564 nm.
Discussion and conclusions

Penetration ability and the distribution of spiropyran in skin

Spiropyrans do have the ability to penetrate skin tissue, most efficiently when the skin sample is placed in a spiropyran water solution, as could be expected. This is however an irrelevant way of applying the spiropyran to skin, in terms of a future skin cancer treatment.

When placing a drop of a spiropyran water solution on either the pig skin or the mouse the skin, only a small amount of the spiropyran seemed to penetrate the skin. It would be interesting to repeat these experiments using a spiropyran solution with a higher concentration.

The spiropyran did manage to penetrate mouse skin, when applied mixed with a cream, but not pig skin when applied this way. This could be due to the fact that little cuts were made unintentionally in the mouse skin when shaved. Because of these cuts, it was perhaps easier for the spiropyran to penetrate the mouse skin than pig skin, regardless of which way it was applied.

The fluorescence intensity profile measurements of the mouse skin cryosections show that in most of the cases there is higher fluorescence intensity at the edges of the skin, indicating that there is more of the spiropyran there. At the same time, there is also high auto fluorescence from the skin surface, making the results in this type of analysis quite difficult to interpret.

Although not analyzed in this project, an interesting result seen in many of the mouse skin cryosections was that the spiropyran seemed to prefer the hair follicles. This could open up for the possibility of treating skin tumors which are close to or in contact with hair follicles using the spiropyran.

Skin quality

When cutting cryosections of the pig skin, it would often be in too poor condition to stay intact; it would get torn apart when cutting. If experiments like the ones described in this project using pig skin were to be repeated, it would therefore be a good idea to use as fresh pig skin as possible. This was also the reason why mouse skin was used: it was quite easy to get a hold of fresh mouse skin.

Human skin models

Although mice are often used in medical research to try to answer questions about human physiology and human diseases, it is questionable if mouse skin is suitable as a model for human skin. It is much thinner than human skin (mouse skin is usually about a few hundred micrometers thick, like the thinnest human skin), and it is also, unlike human skin, covered with fur. Even if the skin is shaved, although it’s preferable not to shave since this might damage the skin samples, the issue with its thickness not being that of human skin still remains.

Pig skin has proved to be a quite good model for human skin. It is closer in thickness to human skin and like human skin also has sparse hair coverage. Unfortunately, in the time frame of this project, it was difficult to get a hold of fresh pig skin, fresh meaning a few hours after the animal had died.
Viewing skin parallel to the skin surface and viewing skin cryosections

If the aim is to study how deep into skin the spiropyran can penetrate and the distribution of the spiropyran in skin, it is better to, in a microscope, view skin as cryosections. In principal, the study could also be carried out with a confocal microscope for skin samples viewed parallel to the skin surface. The confocal microscope could be used to generate a 3D image, and in this way it would be possible to view the skin from the surface and some distance into it. This was also the original intention for this project. However, when treating skin samples with the spiropyran as described in this project, it could be expected that there would be more of the spiropyran at the edges of the skin samples, because this is part of the samples the spiropyran first comes in contact with. Also, the closer to the edges the spiropyran is, the easier it would be to detect it with UV radiation. This way of studying the spiropyran in skin could therefore give a false image of the distribution of the spiropyran from the skin surface and some distance into the skin. In this sense viewing skin cryosections in a microscope for detecting the spiropyran is superior to viewing skin samples parallel to the skin surface in a confocal microscope: no matter at what depth the spiropyran is in the skin, it has an equal chance of being detected with the UV radiation.

It is also standard when viewing skin in a microscope to cut skin cryosections before. This way of viewing makes it easier to see the different skin layers, and therefore, as in this project, easier to see which layers of the skin the spiropyran "prefered". When viewing skin samples parallel to the skin surface, it was very difficult to say what the different structures seen were.

Franz cell

Results from the Franz cell experiment, showed that the spiropyran did not seem to manage to penetrate all the way through the skin used. However, this is not necessarily a negative result when the goal for a future skin cancer treatment would be to get the spiropyran to stay in the skin. It was also investigated if the spiropyran was in fact stuck in the skin, but unfortunately it could not be a hundred percent confirmed that this was the case. If the Franz cell experiments were to be repeated, further investigations of the skin exposed to the spiropyran could be made, for example by cutting cryosections to search for the spiropyran in these.

On the other hand, if the spiropyran did not penetrate the skin at all, this could have been a result of the amount of the spiropyran in the skin cream applied to the skin that was in its MC form. No measurements were made before the Franz cell experiment to measure the amount of MC and SP in the spiropyran water solution mixed with the skin cream. If too much of the spiropyran in the solution was in its MC form, this could have made it more difficult for the spiropyran to penetrate the skin, since the MC form is polar while the first layer of the skin, stratum corneum, is hydrophobic. Additional experiments like these could be made where as much as possible of the spiropyran is in its non-polar SP form before applying it to the skin surface.
**Future work**

If continuing the work described in this project, it would first and foremost be interesting to do a larger number of the cryosection-experiments, for more reliable results. Repeating these experiments, using pig skin, would be a good idea, since pig skin is a good model for human skin. And if possible, also try to get a hold of more fresh pig skin, where the skin is treated with the spiropyran only a few hours after the pig has died and the skin been removed.

The Franz cell experiment could also be repeated, but instead of applying a spiropyran cream to the skin, a spiropyran water solution could be used. Maybe the spiropyran would more easily penetrate the skin this way.

The spiropyran seemed to penetrate mouse skin much better than pig skin. Besides mouse skin being much thinner than pig skin, another difference between the two skin tissue types in this project was that cuts had been made in the mouse skin when shaved, and not in the pig skin since it was never shaved. Perhaps a good way to distribute the spiropyran to skin is via cuts? For this reason, future experiments could be made where the spiropyran is applied through for example thin needles at specific locations in the skin. The distribution of the spiropyran could then be studied over time, to see if the spiropyran would stay where it was applied.

In order to study the cytotoxicity of the spiropyran for skin cells, experiments described in the abstract of this project, cited from the reference 3-article, could be repeated using skin cells (keratinocytes) instead of HEK293 cells.

Skin models of living skin could also be treated with the spiropyran. Epi skin, which is artificial human epidermis, grown from human skin cells, could be used in such experiments.

Although there are lots of different experiments that could be carried out to see how well the spiropyran penetrates skin tissue and/or study the cytotoxicity of the spiropyran in skin cells, the ultimate goal is however to investigate the behavior of the spiropyran in skin tumors, which was not done in this project. On the other hand, tumor cells often mimic the cells from which they originate, so results of the behavior of the spiropyran in non-cancerous skin could also be an indication of its behavior in skin tumors. Therefore, the experiments carried out in this project (using non-cancerous dead skin tissue) are still interesting and meaningful to do.

If however experiments were made where the spiropyran was applied to skin tumors, a first step could be to try to get a hold of human skin tumors that have surgically been removed, as treatment, and apply the spiropyran to these, then view them in a microscope.

In the end, if possible, experiments could also be made using living animals that have skin tumors, for example mice, to see if skin cancer is treatable using the spiropyran and UV radiation.
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Eva Hjelm

Others
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