Assignment: Culture conditions [Temperature]

Cells are cultured in many ways, depending on aspects like cell type and research goals. Although the most favourable environment for cells can change per species, it is important that mammalian cells grow in a physiological temperature of 37 ^{o}C. At this temperature, the cells grow most efficient, while having an optimal enzymatic activity and protein synthesis. Consequently, a shift in temperature can negatively impact the cell culture not only functionally but also morphologically (form or shape). In this exercise, the effects of such a 'temperature shock' on your cells will be visualized.

	Questions	**Answer / Notes**
1	What will happen inside the cells when they are in an environment without this physiological temperature?	
2	What are the morphological consequences of a 'temperature shock'?	
3	Can you think about different activities inside the lab that could induce a temperature change?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cultures into two separate culture vessels in the recommended cell density.	
3	Place one vessel underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2) **(Group 1)**.	
4	Place the other vessel underneath the CytoSMART Lux 2 microscope and place on a workbench (~24 ^{o}C) **(Group 2)**.	
5	Start an experiment with the CytoSMART software and end after the culture has reached confluency.	
6	After the experiment has ended, check the CytoSMART Cloud for data and images.	
7	Can you see the effects of a change in temperature on cell morphology, viability and behaviour? Think about all the actions that must be taken outside of the incubator and its effect on your culture.	

Footnote: **If you have the possibility to use* <u>*two*</u> *Lux 2 microscopes, create* ***Group 1*** *and* ***Group 2*** *at the same time. Otherwise, create the groups separately one at a time.*

Assignment: Culture conditions [Environment]

Besides the culture temperature, the cellular environment should contain a medium that consists of the essential nutrients, growth factors and hormones for the cell type of interest. These factors will ensure that the cells are provided with the right ingredients to stay healthy. Different cell types require specific nutrients and growth factors. This exercise will show what happens to cells when they are not provided with the medium containing these essential factors.

	Questions	**Answer / Notes**
1	What are the most important components that culture medium should contain?	
2	Pick a specific adherent cell type; what are the essential ingredients these cells need to stay healthy?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cultures into two separate culture vessels in the recommended cell density. ○ Add cell specific recommended medium to one culture **(Group 1)**, and add medium without growth factors (e.g. FBS) to the other cell culture **(Group 2).** **	
3	Place the vessels underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
4	Start an experiment with the CytoSMART software and end after the culture has reached confluency or another appropriate time frame.	
5	After the experiment has ended, analyse the cell proliferation with the confluency algorithm in the CytoSMART Cloud.	
6	Can you see differences between the two cell cultures regarding their growth pattern and morphologic characteristics?	

If you have the possibility to use <u>two</u> Lux 2 microscopes, create **Group 1 and **Group 2** at the same time. Otherwise, create the groups separately one at a time.*
*** You can also try to leave out other essential components in the 'random' medium and investigate its effect.*

Assignment: Culture conditions [Substrates]

There are two types of cells regarding their growth system; as monolayers on a substrate (**adherent cells**) or while floating in suspension (**suspension cells**). Adherent cells make use of an anchor mechanism and therefore must be cultured on a suitable surface that is treated to allow for cell adhesion. When designing an experiment with adherent cells, it is especially important to choose the right artificial substrate. This exercise will show the differences between treated and non-treated culture substrates and its effect on consequent cell morphologies.

	Questions	**Answer / Notes**
1	For adherent cells; what are the requirements regarding their culture environment?	
2	What would happen if the culture environment does not fulfil these requirements?	
3	Pick a specific adherent cell type; what culture vessel should be used when culturing these cells?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cells into a new culture vessel in the recommended cell density. ○ Pipet one cell solution into a tissue culture treated (**Group 1**) and one cell solution into a non-tissue culture treated vessel (**Group 2**).	
3	Place the vessels underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{0}C + 5% CO_2).	
4	Start an experiment with the CytoSMART software and end after the culture has reached confluency.	
5	After the experiment has ended, analyse the cell proliferation with the confluency algorithm in the CytoSMART Cloud.	
6	Can you see differences between the two cell cultures regarding their growth pattern and morphologic characteristics?	

If you have the possibility to use two Lux 2 microscopes, create **Group 1 and **Group 2** at the same time. Otherwise, create the groups separately one at a time.*

Assignment: Culture conditions [Contamination]

Sterile culture conditions are crucial for a proper cell culture. However, these sterile conditions can be affected when contamination takes place. It is hard to determine the cause of contamination, but it commonly relates to improper work techniques, used materials and reagents or dysfunctions of equipment and facilities. Once contamination has taken place, it is almost impossible to sterilize the culture and proceed with the research. Therefore, it is important to know how you can prevent and recognize contaminations within your culture. This exercise will teach you the characteristics of contamination.

	Questions	**Answer / Notes**
1	What are the different sources that can cause a contamination within a cell culture.	
2	How are these sources linked to the causes of contamination?	
3	What is commonly used to reduce the risk of contamination?	
4	How can you recognize contamination within your culture vessel? Try to describe per source of contamination.	

	Exercises:	**Notes**
1	Take a healthy culture of a specific adherent cell type in a culture **flask**.	
2	Pass the cells into a new culture vessel in the recommended cell density. ○ Use culture medium that does **not** contain Pen-Strep.	
3	Take a pipet tip, make sure to touch it with your bare hands and put it in the culture vessel.	
4	Place the vessels underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{0}C + 5% CO_2). ○ Make sure not to use a public incubator and clean properly afterwards.	
5	Start an experiment with the CytoSMART software and end after you recognize the answer to **Question 4** in your culture vessel.	
6	After the experiment has ended, check the cloud for data and images.	
7	Can you identify any source that could indicate a contamination? And how does the medium in the culture vessel looks like?	

Assignment: Cell behaviour [Aging]**

Cells go through a life cycle that includes growth, maturity and eventually death. This aging cycle of cells often ends with a process called **senescence**. Senescence is a stage in the aging cycle where cells lose their ability to divide. In research it is extremely important to use cells that are still capable of dividing, to generate the most reliable results. Therefore, it is important to use cells that are not passaged for an extensive amount of times, depending on the cell type used. In this exercise, the influence of high passage numbers on a cell's ability to grow and divide will be elucidated.

	Questions	**Answer / Notes**
1	Pick a cell type that you want to use for this experiment. What is the recommended maximum passage number of these cells?	

	Exercises*:	**Notes**
1	Take one healthy culture of this cell type that has already passage number above 10.	
2	Pass them into a new culture vessel in the recommended cell density (**Group 1**).	
3	Take one healthy culture that has just been thawed (or thaw them yourself and passage them at least a week before this experiment) of the same cell type as in **Exercise 1**.	
4	Pass them into a new culture vessel in the recommended cell density (**Group 2**).	
5	Place the vessels underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
6	Start an experiment with the CytoSMART software and end after the culture has reached confluency.	
7	After the experiment has ended, check the CytoSMART Cloud for data and images.	
8	Can you identify the effect of cell aging in your cultures? Think about how this can affect the cell response if you want to perform experiments.	

If you have the possibility to use <u>two</u> Lux 2 microscopes, create **Group 1 and **Group 2** at the same time. Otherwise, create the groups separately one at a time.*

*** If you have to thaw cells yourself, make sure to thaw new cells a week before starting this experiment.*

Assignment: Cell behaviour [Cell death]

Cellular death is an essential mechanism for an organism to grow and survive. However, there are two different ways in which a cell can die; apoptosis and necrosis. Apoptosis is a regulated variant of cell death, while necrosis is an unregulated type of cell death. Both these mechanisms are stimulated by different signals; apoptosis is regulated inside the cell, while necrosis is initiated by an external stimuli. Moreover, apoptosis makes sure that unwanted cells are removed without causing initiating a immune response, while in necrosis inflammatory cellular contents are released. One visible change that differs between to two types is the way of cell destruction. In this exercise, the visual difference between these two concept will be elucidated.

	Questions	**Answer / Notes**
1	Make sure to have two cell cultures that are healthy and are ready for passage. (Ask a supervisor to be sure)	
2	How is apoptosis induced in the specific cell type in the culture?	
3	How is necrosis induced in the specific cell type in the culture? (make sure that this one is available for you)	
4	What are the differences between the two mechanisms that could be visualized under a brightfield microscope?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cells into two separate new culture vessels in the recommended cell density. ○ Make sure to induce apoptosis in one vessel (**Group 1**) and necrosis in the other vessel **(Group2).**	
3	Place the vessels underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
4	Start an experiment with the CytoSMART software and end after the recommended time to visualize the process.	
5	After the experiment has ended, check the CytoSMART Cloud for data and images.	
6	Now try to identify the visible differences between the two cellular death mechanisms.	

Note: *If you have the possibility to use two Lux 2 microscopes, create **Group 1** and **Group 2** at the same time. Otherwise, create the groups separately one at a time.*

Assignment: Cell behaviour [Migration]

Cell migration is an essential process in the development and maintenance of organisms. All kind of physiological and pathological processes, like tissue formation, wound healing and immune responses require movement of cells in specific directions. These movements are initiated by certain external stimuli. Errors in cell migration responses can lead to negative consequences like for example; vascular diseases, tumor formation and metastasis. A good understanding of the fundamental mechanism of cell migration may thus lead to the development of innovative therapeutic strategies. In this exercise, the cell migration principle will be investigated.

	Questions	**Answer / Notes**
1	What are the different stimuli that activate cell migration?	
2	What are the different mechanisms that a cell uses to migrate?	
3	How can these various forms of cell migration be induced in a culture that contains adherent cells?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Make sure to create a scratch inside the culture vessel by scraping a pipet tip over the surface (**Group 1**).	
3	Place the vessels underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{0}C + 5% CO_2). (Make sure to have the scratch in the focus area)	
4	Start an experiment on the CytoSMART software and end after the recommended time to visualize the process.	
5	After the experiment has ended, check the CytoSMART Cloud for data and images.	
6	Also try to induce cell migration by using another external stimulus **(Group 2).** (e.g. rigidity gradients)	
7	Try to identify the morphological changes a cell undergoes when it is migrating over the surface.	

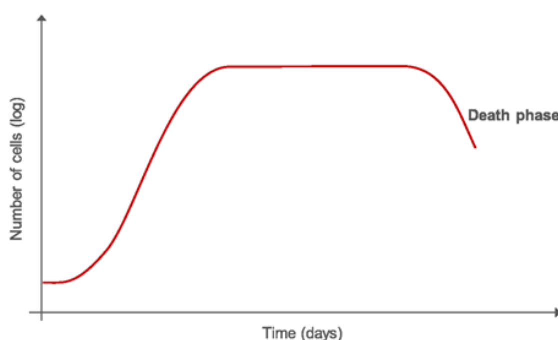
If you have the possibility to use two Lux 2 microscopes, create **Group 1 and **Group 2** at the same time. Otherwise, create the groups separately one at a time.*

Assignment: Cell cultures [Sub culturing]

Sub culturing or passaging is the removal of old medium and providing the cell culture with fresh medium. The growth of cells in a culture proceeds in different phases. During the first phase cells proliferate exponentially. When 80% confluency is reached (in case of adherent cells) or when the culture medium does not have the capacity to support any further cell growth (in case of suspension cells), cell proliferation is attenuated. The cells transit into the second phase, the stationary phase, which is followed by cell death. To keep cells at an optimal density for continued growth and proliferation, cells should be passaged at the right time in the growth curve. This exercise will provide an approach to precisely determine the optimal passage point.

	Questions	**Answer / Notes**
1	What are the different phases a cell undergoes in between passages?	
2	What would be the ideal timepoint within this cycle to subculture your cells?	

	Exercises:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cells into a new culture vessel.	
3	Place the vessel underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
4	Start an experiment on the CytoSMART software and end after the recommended time to reach full confluency.	
5	After the experiment has ended, analyze the cell proliferation with the confluency algorithm in the CytoSMART Cloud.	
6	Try to find the optimal timepoint in the confluency graph to subculture of pass the specific cell type that you use.	



Assignment: Cell cultures [Passing point]

The passing point of cells is extremely important to ensure that cells are ready for an experiment on a particular day or to maintain cell cultures for future actions. Regarding the growth curve, it is important that cellular processes like proliferation are undisturbed as much as possible. In this exercise, it will be visualized what happens to cells when they are passed either too soon or too late.

	Questions	**Answer / Notes**
1	At what timepoint in the growth curve are cells in optimal state to be used? How can this state be recognized by brightfield imaging?	
2	Pick a cell type and look for a growth curve. What is the timepoint when cells are not ready for passaging? What is the optimal timepoint to pass these cells?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cells into two new culture vessels in the recommended cell density.	
3	Pass the cells of one culture vessel before they reach the log phase (**Group 1**). ○ Pass the cells of the other culture vessel after they passed the stationary phase (**Group 2**).	
4	Place the vessel underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
5	Start an experiment on the CytoSMART software and end after the recommended time to reach full confluency	
6	After the experiment has ended, analyze the cell proliferation with the confluency algorithm in the CytoSMART Cloud.	

If you have the possibility to use <u>two</u> Lux 2 microscopes, create **Group 1 and **Group 2** at the same time. Otherwise, create the groups separately one at a time.*

Assignment: Cell cultures [Cell density]

Cell viability and health is not only influenced by culture conditions like the chemical environment or temperature but is also determined by their spatial surroundings. In other words, the space that is preserved for one single cell can significantly affect the cell's growth and health. Cells stop growing when they reach full confluency. Consequently, cell density has a high influence on the quality of a cell culture experiment. This exercise will demonstrate what happens to cells when cultured in a low cell density compared to a higher cell density.

	Questions	**Answer / Notes**
1	What can happen to cells when they are seeded in too high/low numbers per culture area?	
2	Consider an adherent cell type that you want to culture and investigate what the recommended seeding density is for these specific cells?	

	Exercises*:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cells into a new culture vessels in the recommended cell density.	
3	Seed them in the recommended seeding density in one culture vessel (**Group 1**)	
4	Seed them in a higher seeding density in another culture vessel **(Group 2)**	
5	Place the vessel underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{0}C + 5% CO_2).	
6	Start an experiment on the CytoSMART software and end after the cells have passed full confluency.	
7	After the experiment has ended, analyze the cell proliferation with the confluency algorithm in the CytoSMART Cloud.	
8	Investigate the time after full confluency is reached. Considering the stationary phase of the cell cycle, do you see the effects that you found by Question 1?	

If you have the possibility to use* <u>*two*</u> *Lux 2 microscopes, create* ***Group 1* *and* ***Group 2*** *at the same time. Otherwise, create the groups separately one at a time.*

Assignment: Research specific [Cytotoxicity]

Especially within the field of tissue engineering and drug design, cytotoxicity is an important factor to consider. Cytotoxicity investigates the potential toxicity of a designed drug or material for cells. This toxicity often comes from biologically active compounds in the material. For a successful development of therapeutic agents, it is crucial to produce minimal to no cytotoxic side effects. For this reason, cytotoxicity studies are extremely important in research. In this exercise, the potential cytotoxic effects of certain chemicals will be shown.

	Questions	**Answer / Notes**
1	What can happen to cells when they are in contact with toxic compounds?	
2	How could these effects be visualized by brightfield microscopy?	
3	Pick a specific cell type that you want to use and try to find a material that is toxic for these cells.	

	Exercises:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Add a toxic material (that you have found in **Question 3**) to the cell culture.	
3	Place the vessel underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
4	Start an experiment on the CytoSMART software and end after the material has affected the cells.	
5	After the experiment has ended, check the CytoSMART Cloud for data and images.	
6	Did something happen to your cells? Think about the relevance of cytotoxicity experiments for drug development or tissue engineering.	

Assignment: Research specific [Molecular concentration]

Osmosis is a process between compartments where there is a net movement of water over a (semi-)permeable membrane into a area with a higher solute concentration than it came from. Osmosis is a process that happens in tissues to maintain a constant environment in the body. In cell cultures, it therefore important to mimic the physiological environment when predicting cellular responses. Osmosis takes place until the more concentrated solution (hypertonic) has come to an equilibrium with the less concentrated solution (hypotonic). In case of cell cultures it is therefore important to consider whether the environment in hypertonic or hypotonic compared to the intracellular milieu.

	Questions	**Answer / Notes**
1	Which direction would water go if the intracellular milieu is hypotonic compared to the extracellular milieu? And what if it was the other way around?	
2	What would happen with the cells in both situations?	
3	What is an example solution that would be very hypertonic compared to cells? And which solution would be hypotonic compared to cells?	

	Exercises:	**Notes**
1	Take a healthy culture of a specific adherent cell type.	
2	Pass the cells into a new culture vessel.	
3	Add a hypotonic solution to the cells.	
4	Place the vessel underneath the CytoSMART Lux 2 microscope and place in the incubator (37 ^{o}C + 5% CO_2).	
5	Start an experiment on the CytoSMART software and end after osmosis should have taken place (2 to 4 hours).	
6	After the experiment has ended, check the CytoSMART Cloud for data and images.	
7	What happened to the morphology of the cells? Imagine what will happen if you add to concentrated solutions in your experiments.	