Isolation of renal endothelial cells from a kidney preservation pump: a problem definition

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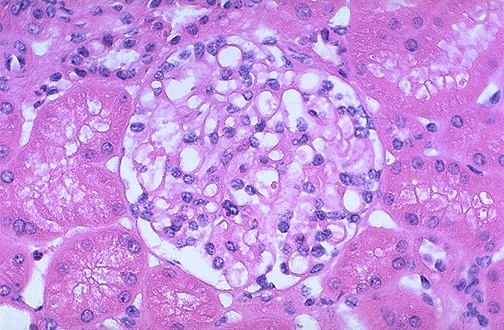


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# Background information

## Introduction

When an adolescent turns 18 in the Netherlands, a letter is sent to the adolescent to submit a registration in the donor codicil. This registration is necessary because there is a shortage of organ donors in the Netherlands, but this problem occurs all over the world. Especially in kidney donors, the transplant rates are low, about 10% of the patients in need of a kidney actually receive one within the time frame the patients benefit from having a new organ. (Hamar, 2018) On March the 30th 2017, there was a waiting list of 629 registered people waiting for a donor’s kidney solely in the Netherlands. (Anon., 2017) In contrary to other organ transplantation methods, there are two different methods to obtain the kidney. The first method is to obtain the kidney from a living donor that has a genetic match to the receiver, usually a family member. This transplantation is planned and the kidney can be transferred from the donor to receiver without any problems because they both will be hospitalized in the same hospital before and at the time of donation. The second option is to obtain the organ from a departed donor. Using this method, delivering the donor organ from the deceased patient to the receiver is a crucial share in the method. The function of the organ and graft survival decrease when the organ is disconnected from a body for a longer time. (Ciancio, 2010) In the case of donation post-mortal, the method of delivery and preservation of the organ come highly into play when studying the success rates of the transplantation.

## Simple Cold Storage

Since 1955, a rise in interest on the subject of the optimization of the transplantation of kidneys has been seen. (Lee, 2009) This has led to the first renal preservation method that was developed; the Simple Cold Storage (SCS). This method is a static preservation method, the fluid that is injected into the organ does not flow through during preservation and the organ is preserved on ice (4 degrees Celsius). This hypothermic temperature causes the reduction of enzyme activity and metabolism by 58%. (Catena, 2013) The solution that is used to store the organ has the same ionic composition as the kidney cells, equal to the osmolarity of 140 mM. (Lee, 2009) This osmolarity, along with glucose in the medium, prevents the cells from swelling and reduces the stresses on the cell membrane. Using this method, the kidney can be stored and transported up to 24 hours after removing it from the donor’s body.

## Hypothermic Machine Perfusion

Although the CSC method functions well and has a graft survival of 90 percent after one year of transplantation (Moers, 2012), research was performed to contrive a better working method to improve the quality of the organ over time. Several methods were developed with a dynamic flow of the medium. Hypothermic machine perfusion (HMP) is the method most used nowadays with a graft survival rate of 94 percent after 1 year. (Moers, 2012) The temperature resembles the temperate used in the CSC method, between 0 degrees Celsius and 4 degrees Celsius. (Catena, 2013) This method is based on the flow of medium through the organ during preservation. The continuous flow supplies the kidney cells of nutrients and oxygen and eliminates all waste and toxicities from the tissue. The kidney can be preserved for up to 36 hours when the HMP is used. (Ciancio, 2010)

## Normothermic Machine Perfusion

Research is performed to observe the effects of normothermic machine perfusion (NMP). This method is based on maintaining the same temperature as in vivo. The metabolic activity is not slowed down, so the kidney functions as it did before it was removed from the body. The solution that runs through the kidney is autologous blood. (Lee, 2009) In research so far, the kidney can be preserved 24 hours longer in comparison to the CSC method. The main problem in CSC and HMP, the incidence of primary non-function of the graft after reheating it is minimalized using the NMP method because the temperature fluctuation of the cells ideally is zero. Although the method has a promising perspective, the challenge is to supply the organ with oxygen as a consequence of an inadequate blood supply because the metabolic activity of the cells is maintained by maintaining the temperature to body temperature. (Hosgood, 2014)

## Research of the group

The transplantation pumps are a good replacement for usage in the preservation of kidneys in transplantation but have other applications. Rosa Lammerts, performing research in the UMCG, has discovered that the medium that flows through the kidney contains endothelial cells after the organ is transplanted. Next to the endothelial cells, also other unknown renal cells run through this medium. The cause of these amounts of cells in the medium is thought to be shear stress on the vessel walls as a consequence of flowing preservation fluid and the pressure of the pump. Besides the direct cause of these cells that are present in the medium, a lot of other information about these cells is unknown, for example, the number of cells in the fluid. As has been proved in experiments, the number of cells in this fluid varies a lot as a result of different circumstances. Different causes can be linked to this observation, the time of preservation and the type of pump can cause the pump to contain more or fewer cells. The goal of the research performed by Rosa is to isolate the endothelial cells to perform experiments concerning HLA typing on. These cells for research can only be obtained from the organs of postmortem donors, as the kidneys are preserved for a longer period of time in contrast to living donors. The HLA typing is based on the antigens that are expressed on the surface of the endothelial cells. These endothelial cells are exposed to the blood of the receiver first after transplantation and form the initial response to the recipient. When the antibodies in the blood of the receiver respond to the antigens on the surface of the cells of the transplanted organ, an immune response is put into motion as a cause to the rejection of the organ by the body. This phenomenon is highly unwanted to occur. To ensure that the chance of rejection of the organ is minimal, the HLA types of the donor and recipient should be the same as much as possible. There is a lot known about this typing, leading to successful renal transplantations, but also a lot is unknown. The endothelial cells that are eventually isolated out of the pumps are used to research these HLA antigens in combination with the blood of the receiver to activate the classical complement system and study the response. More knowledge about the rejection of the organ is gained using this method. The pumps that are used nowadays in the Netherlands are the LifePort and the Kidney Assist. The difference between these pumps is that the LifePort contains a filter that filters a lot of endothelial cells out of the medium while the kidney assist has no such filter. When a pump post-transplantation comes in at any given time, the fluid is kept cold and sterile until the sorting of and plating protocol of the EC’s is performed.

## Morphology of endothelial cell

To be able to study the method to isolate the renal endothelial cells, the characteristics of the cell should be specified. The blood vessel endothelial lining is a highly metabolic structure that differs strongly in phenotype. (Aird, 2007) These endothelial cells form the inner layer of blood and lymphatic vessels. The main function of the endothelium is to create a barrier between the vessel and the peripheral tissue. Responding to signaling molecules, this endothelium changes in morphology to change in permeability. Typically, EC’s are flat and elongated. In certain circumstances, they can become cubical or plump. Several specific endothelial markers are expressed in the cells. There is a difference in endothelial phenotype between different organs. (Aird, 2007)

An EC is recognized by various characteristics. EC’s can be divided into three categories; the arterial, true (exchange between capillaries and tissue) and venous capillary endothelial cells. These cells from different categories have their shape in common; endothelial cells are usually long and hexagonal. (Adamson, 1993) A point of recognition is the cleft lengths of these arterial, true and venous capillary endothelial cells, these cells are respectively 135 ± 28 microns; 98 ± 28 microns and 139 ± 20 microns.

Other endothelial cells that might be present in the preservation fluid are glomerular endothelial cells. These cells form the barrier between the blood vessels and the nephron when the blood is filtered and respond to the amount of fluid that has to be filtered out of the vessel system. (Satchell, 2009) These endothelial cells contain small holes in them called fenestrations to supply a larger exchange between the blood vessel and exterior.

## The current method to isolate the cells

Currently, the method to isolate the endothelial cells is based on the platelet endothelial cell adhesion molecule (PECAM-1), also named the cluster of differentiation 31 (CD31). This molecule is present on the cell membrane of endothelial cells and is responsible for the reciprocal adhesion of endothelial cells. (Lertkiatmongkol, 2016) When the cells are plated, it takes one week for the cells to adhere and to form a monolayer in the well. After this, the isolation can be performed. First, the amount of endothelial cells in the well is estimated. Based on the number of cells, an amount of Dynabeads is applied to the mixture of cells and these beads adhere to the CD31+ cell adhesion molecule of the endothelial cells. Dynabeads are magnetic markers attached to antibodies. When the antibodies attach to the antigen expressed on the cell membrane, the magnetic marker can be filtered out using a magnet. CD31 negative cells are not affected by the magnet and the endothelial cell population is isolated from the fluid. Dynabeads affect the viability of the endothelial cells. A rate of 50 beads per cell is the most accurate when focusing on the isolation of cells (83% ± 5.3), while the amount of 4 beads per cell is found to be at its optimum when the goal is to proliferate the cells for a longer time (± 6 days). (Tiwari, 2003) After the beads procedure is applied, the cells can be plated out and used in experiments or be placed in the freezer for later research.

# Problem definition

## The problem

While the current method of isolating the endothelial cells has a relatively high success rate, it remains to be a labor-intensive and time-consuming process. It takes a long time for the cells to replicate and adhere to the well when they are plated. The plating of the cells and waiting for the cells to adhere to the wells (±1 week) slows down the progress. Before the cells can be used, useful time has passed for the researcher. The interest of the researcher is to perform experiments on these sorted cells as soon as possible. The work is labor-intensive and the initial plating takes 1 hour, the beads procedure around 3 hours and the continuous plating 1 hour. This plating is mostly done by students that work in the lab for the first time and have to learn to work sterile. This inexperience may lead to more infection in the wells. When infection occurs, the cells cannot be used to perform experiments on. Another problem is that when the more the cells are plated onto a new plate and replicate, the more the cells dedifferentiate. As time passes, the renal endothelial cells dedifferentiate back to universal, non-tissue specific endothelial cells. When this happens, research concerning the rejection of kidneys cannot be performed because the effect of the kidney transplant is researched, in contrast to the initial response of the body to the transplanted organ. (Thum, 2000) Another problem with the beads procedure is that the marker CD31 is also expressed in leucocytes and platelets. (Aird, 2007) When the procedure is applied, the leucocytes and platelets that are present in the cell suspension will not be filtered out of the suspension. The CD31+ molecule functions as a trigger to downstream inhibitory signaling during cell-cell interactions and plays a part in T-cell activation and angiogenesis. (Liu, 2012 ) The problem of affected viability will not be represented in the cause-effect diagram but will be taken into account in the requirements and wishes as the project will focus on the plating of the cells and not the beads procedure itself.

## Stakeholders

Only stakeholders that are directly involved in this problem will be taken into account when designing a new method. These stakeholders are the researcher, the employees of the researcher and the head of the lab.

The stakeholder that is affected the most by the time-consuming process and labor-intensive work is the researcher. The research of the researcher will have a faster progress if the time of sorting is minimalized. This researcher focusses on fast process and results and prefers a sorting process that is as fast and precise as possible. Next to the researcher, the employees of the researcher, mostly students, need to be taken into account as well. Employees help the researcher with the plating of the cells but do have their own results from the research. They prefer to perform an automated solution because this is less intensive work and the students are less likely to infect the cells what is stimulating for the progress of the researcher and themselves. The researcher has to take the head of the lab into account because not every solution at each expense is possible. The head of the lab prefers a solution to this problem that is reusable to save money and also prefers a solution that does not take up a lot of space. Every possible solution will have to pass him/her to be realized. The researcher and head of the lab have opposing interests that might form a difficulty in this project.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Stakeholder** | **Characteristics** | **Expectations for solution** | **Difficulties contributing to problem** | **Influence on project** |
| **The researcher** | Plating the cells inhibits the process of the research because of time issues. Spending time on plating also means less time for research. | A method that sorts the cells quick and accurate. The method needs to be easily accessible because it should be performed after working hours without supervision. | Wants to make fast process, but a new protocol might lead to a period of tryouts and fails. This inhibits the progress and is at the expense of useful pumps. | The new protocol will have the most influence on the research and eventually results of the researcher. |
| **The employees of the researcher** | Employees/Students have to perform labor-intensive work. They prefer an automated procedure that is time-saving. | Automated method that is easily accessible and learnable. | Need to be trained if a new protocol is applied. | The method needs to be easily accessible because the employees change quickly. |
| **The head of the lab** | Beads cannot be reused. Space in the lab is expensive and not always available. Also, the purchase of machines can be expensive. | Less expensive option, reusable method  A compact method is preferred. | Can inhibit the progress of the solution by not allowing the expense that will be made as an investment of the sorting. | Every solution should be checked with the head of the lab because he/she has a decisive voice. |

Table 1 Stakeholders

## Cause-effect diagram problem

Cause

The renal endothelial cells need to be plated to perform a CD31+ beads procedure

Cells need to be plated several times

The renal endothelial cells dedifferentiate to general endothelial cells

Cells cannot be used for research

Cells are more prone to infection

Time spend by the researcher can be used better otherwise

Labor is expensive

Effect

Figure 2 Cause-effect diagram of the problem

Slow progress

Plating the cells is labor-intensive

Plating the cells is time consuming

# Goal

The goal of the new method is to filter the renal endothelial cells out of the medium that is obtained from the perfusion machine after kidney preservation. This method should be more sufficient then the method used nowadays, in the field of time and labor intensity. The problem will be tackled at the base; the plating of the complete fluid that runs through the organ. The beads procedure will be changed or replaced by a faster, less labor-intensive method. Preferably, the method should filter out the renal EC’s without any plating to prevent the cells from dedifferentiating. After filtration, the cells should be viable and replicate. The cells should be able to be used in experiments and eventually immortalized as well. The filtration itself should not take too long because the pumps can come in at any time and the employees should be able to process at the time of arrival. This is also why it is important to deliver a relatively easy procedure that can be executed after working hours of the technicians. Eventually, this improvement in selection progress will lead to faster progress in the field of kidney transplantation.

There might be a chance that not all three problems caused by the plating of the cells can be solved by targeting this plating of the cells. Minimalizing the time that it takes to isolate the EC’s is the main target in this project.

## Cause-effect diagram goals

Cause

The renal endothelial cells do not need to be plated to perform a selection process

The renal endothelial cells do not dedifferentiate to general endothelial cells

Cells can be used for research

Cells are not prone to infection

Researcher can spend time on more important work

Expensive labor is not necessary

Effect

Figure 3 Cause-effect diagram of the goals

Fast progress

Plating the cells is not labor-intensive

Fast method

# Design assignment

Several strategies can be used to realize the goal. Each strategy is a design assignment:

1. One possibility is to realize a protocol of an automated machine. A protocol needs to be tested to be idealized. A protocol should include all the actions and the time that it takes to carry out this protocol. Also, a template of the machine might be included. Sorting machines place every cell on a spectrum and the right areas on this spectrum need to be found and marked to filter the cells out.
2. Another possibility is to realize an automated version of the protocol already existing. The beads work quite well and turning the existing protocol around to save time is an option to save time and labor.
3. All possibilities of sorting will be researched, but if none is sufficient a new method of sorting can be realized. Sorting can be done using many properties of cells and not all of them have been concluded in a method of sorting. Researching a new method can be a solution to the time-consuming process.
4. A more rigorous method is to redesign the whole method of doing experiments on the EC’s. It might not be necessary to filter the EC’s out of the medium.

To achieve the goal it might be necessary to distinguish the other cells in the fluid. For as long as the kidney preservation pumps are used in research, only the EC’s are used. Initially, it was not expected for these EC’s to be present in the medium, but other useful cells might be present in the medium as well. In this research, the focus is initially on the filtration of renal EC’s but if possible, the other cells can be filtered and used in research. Also, being able to count the cells at the time of filtration would be a method to achieve the goal. This way, a more clear image of the fluid after preservation of the kidney can be obtained and the method is more effective.

## Demarcation

The viability of the cells and the amount of cells is partially determined by the pump used for preservation because of the stresses on the cells. This use cannot be changed and until the preservation fluid is in the cold storage room, nothing can be changed to the progress. Until this point, the focus is on the kidney itself and the fluid is a product that is accidentally produced along with this process. Even if one of the two pumps eventually turns out to contain the most EC’s, this is not regulated by the researcher. An arrangement with the UMCG team is settled and they determine if the pumps can come in. Sometimes the pump fluid is infected by touching the inside of the preservation pump. The fluid cannot be used if the fluid is infected.

The quantity of the pumps that come in is also not regulatory. This quantity depends on people who die and are registered as a donor as well. When the method is tested, it might be possible that the experiments cannot be performed in the time frame of this project due to a lack of pump fluid. Also, other methods to obtain the renal endothelial cells are excluded. Renal endothelial cells can be obtained directly from the kidney, but a kidney is needed to do so. A single kidney might be donated to science, but the amount needed to perform experiments on can only be obtained as a by-product.

# List of requirements

A list of requirements and wishes is needed to accomplish the goal of finding a new method. If the new sorting method refrains from one of these requirements, the goal is not accomplished. The wishes need to be taken into account but do not form a restricting factor in the designing of a new method/protocol. The requirements that do not state a source are an educated guess or a requirement obtained from the researcher or lab.

The requirements are split up into different categories. The use requirements are the requirements the product needs to have to function properly. The safety requirements are also very important when working in the lab. The MT-II rules need to be obeyed. The most important requirement is that the new method should be sterile. If the cells are infected, they are useless.

## Use requirements:

* The input of fluid should be sufficient to sort all the cells in the medium
* The method should lead to a success rate at least of 80 percent
* The viability of the cells should not be affected negatively
* The device should filter the kidney endothelial cells
* The method should be accessible for students to learn in one day of training
* The method should be able to be carried out outside of working times without supervision
* The heterogeneity of the renal EC should not be lost during the procedure
* The shear stress should not affect the cells
* The temperature should be in between 0 and 38 degrees Celsius
* The shear stress should not affect the viability

## Safety requirements:

* The new method should not modify the DNA of the cells
* The new method should be sterile
* The new method should keep the cells from spreading
* The procedure should be in accordance with MT-II lab rules
* The procedure should not be a risk to the researcher working in the lab

## Ergonomic requirements:

* The new method should weigh less than 1 kg when it is performed by hand
* The new method should have no edges that are difficult to clean and kept sterile

## Space requirements:

* The new method should fit inside the laboratory
* The device should not be larger than a fume cupboard

## Time requirements:

* The procedure should take no longer than 3 hours
* The time between the pump and the procedure should be no longer than 36 hours\*

## Wishes:

* The procedure should be sustainable
* The procedure should be reusable
* The procedure should have no waste
* The new method should take as less space as possible
* The new method should take as less time as possible
* The new procedure should be automatic
* All endothelial cells should be filtered out of the medium\*
* The device should be able to identify other cells in the medium\*
* The new procedure should take as less time as possible
* The new procedure should be reproducible in an experimental setting\*
* The number of cells in the medium should be counted
* The viability should be affected as less as possible
* The temperature should be the same as in the body

\* = requirements obtained by Rosa Lammerts

# Analytic Hierarchy Process

The analytical hierarchy process (AHP) is used to decide which requirements or wishes are most important in the process of designing a new method. The AHP calculates a priority factor for each category for each stakeholder. These priority factors often contradict each other because all stakeholders have other interests. For example, the importance of the finances is of little importance to the researcher but is the focus point of the head of the lab.

## Pre-concept selection synthesis I

The pre-concept selection of synthesis I is performed using the most important requirements.

### Researcher

For the researcher, the efficiency of sorting is the most important because all cells need to be sorted out of the fluid. The more cells, the more experiments can be performed. The ease of use and time of the procedure are important as well because the progress of the research is affected by these factors as well.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Ease of use | Use efficiency | Safety | Space in the lab | Time of procedure |
| Ease of use | 1 | 0.33 | 1.33 | 5.00 | 1 |
| Use efficiency | 3 | 1 | 2 | 10 | 1.33 |
| Safety | 0.75 | 0.5 | 1 | 1.33 | 0.5 |
| Space in the lab | 0.25 | 0.2 | 0.75 | 1 | 0.5 |
| Time of procedure | 1 | 0.75 | 2 | 2 | 1 |
| **Priority** | **0.23** | **0.37** | **0.12** | **0.08** | **0.20** |

Table 2 AHP researcher

### Employees of the researcher

The focus of the employees is on the ease of use and time of the procedure. The safety is also important for their own risk while sorting. The efficiency of the sorting is not their priority because they do not focus on the results of the research.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Ease of use | Use efficiency | Safety | Space in the lab | Time of procedure |
| Ease of use | 1 | 2 | 1.5 | 4 | 1 |
| Use efficiency | 0.5 | 1 | 2 | 2 | 0.5 |
| Safety | 0.66 | 0.5 | 1 | 2 | 0.5 |
| Space in the lab | 0.25 | 0.5 | 0.5 | 1 | 0.25 |
| Time of procedure | 1 | 2 | 2 | 4 | 1 |
| **Priority** | **0.29** | **0.18** | **0.14** | **0.08** | **0.31** |

Table 3 AHP employees

### Head of the lab

The head of the lab has his/her focus on the space in the lab and safety requirements. The procedure needs to follow the MT-II rules. When the procedure takes a short time, there is more time for the others in the lab to do their laboratory work. An easy protocol will lead to fewer mistakes by the students, so the lab will function better.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Ease of use | Use efficiency | Safety | Space in the lab | Time of procedure |
| Ease of use | 1 | 2 | 0.25 | 0.25 | 0.5 |
| Use efficiency | 0.5 | 1 | 0.2 | 0.2 | 0.33 |
| Safety | 4 | 5 | 1 | 1 | 3 |
| Space in the lab | 4 | 5 | 1 | 1 | 3 |
| Time of procedure | 2 | 3 | 0.33 | 0.33 | 1 |
| **Priority** | **0.08** | **0.03** | **0.37** | **0.37** | **0.16** |

Table 4 AHP head of the lab

### General weight factor

The weight factor for the importance of each section is calculated by averaging the priorities of each stakeholder. The weight factors will be used during the pre-concept selection used during the designing process. The weight factors of all categories are almost similar, so the categories have the same priority when the designing process is executed.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Ease of use | Use efficiency | Safety | Space in the lab | Time of procedure |
| Researcher | 0.23 | 0.37 | 0.12 | 0.08 | 0.20 |
| Employees of the researcher | 0.29 | 0.18 | 0.14 | 0.08 | 0.31 |
| Head of the lab | 0.08 | 0.03 | 0.37 | 0.37 | 0.16 |
| **Weight factor** | **0.20** | **0.19** | **0.21** | **0.18** | **0.22** |

Table 5 AHP general weight factor

## Pre-concept selection synthesis II

The pre-concept selection of synthesis II is performed using the most important wishes.

### Researcher

Whether or not the method is re-usable matters, but is less important than the other wishes. The identification of the other cells is a project Rosa (the researcher) would like to continue, leading to the high rate of identification in comparison to the other wishes. Also, the number of cells is an interesting fact for the continuation of her research. The viability of the cells is directly linked to the amount of cells that are plated effectively. When the viability is higher, the number of useful cells is also higher.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Re-usable | Identify/sort other cells | Cells counted | Automated procedure | Viability effected |
| Re-usable | 1 | 0.25 | 0.33 | 0.66 | 0.33 |
| Identify/sort other cells | 4 | 1 | 2 | 2 | 1 |
| Cells counted | 3 | 0.5 | 1 | 2 | 0.66 |
| Automated procedure | 1.5 | 0.5 | 0.5 | 1 | 0.57 |
| Viability effected as less as possible | 3 | 1 | 1.5 | 1.75 | 1 |
|  | 0.08 | 0.30 | 0.21 | 0.12 | 0.24 |

Table 6 AHP researcher wishes

### Employees of researcher

For the employees/students, the most important wish is to have an automated procedure. When an automated procedure or template is used, the chance of things going wrong is minimal. Because their own research is affected by the results of the cells. A re-usable method is easier because the students do not have to be concerned about reordering new materials.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Re-usable | Identify/sort other cells | Cells counted | Automated procedure | Viability effected |
| Re-usable | 1 | 2 | 2 | 0.33 | 0.5 |
| Identify/sort other cells | 0.5 | 1 | 1 | 0.25 | 0.5 |
| Cells counted | 0.5 | 1 | 1 | 0.25 | 0.5 |
| Automated procedure | 3 | 4 | 4 | 1 | 2 |
| Viability effected as less as possible | 2 | 2 | 2 | 0.5 | 1 |
|  | 0.17 | 0.10 | 0.10 | 0.41 | 0.22 |

Table 7 AHP employees wishes

### Head of the lab

The head of the lab is especially interested whether the method is re-usable. Less waste is created and when a method is re-usable, fewer costs are made. The investment will be done one time, instead of on a regular base. When the protocol is automated is also important for the head of the lab because this will shift the number of employees in the laboratories. The viability of the cells is not very important to the head of the lab, but more important than the identification and counting because keeping the viability high will lead to more progress.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Re-usable | Identify/sort other cells | Cells counted | Automated procedure | Viability effected |
| Re-usable | 1 | 4 | 4 | 2 | 3 |
| Identify/sort other cells | 0.25 | 1 | 1 | 0.5 | 0.5 |
| Cells counted | 0.25 | 1 | 1 | 0.5 | 0.5 |
| Automated procedure | 0.5 | 2 | 2 | 1 | 1.5 |
| Viability effected as less as possible | 0.33 | 2 | 2 | 0.66 | 1 |
|  | 0.41 | 0.10 | 0.10 | 0.21 | 0.17 |

Table 8 AHP head of the lab wishes

### General weight factor

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Re-usable | Identify/sort other cells | Cells counted | Automated procedure | Viability effected |
| Researcher | 0.08 | 0.30 | 0.21 | 0.12 | 0.24 |
| Employees of the researcher | 0.17 | 0.10 | 0.10 | 0.41 | 0.22 |
| Head of the lab | 0.41 | 0.10 | 0.10 | 0.21 | 0.17 |
| **Weight factor** | **0.22** | **0.17** | **0.14** | **0.25** | **0.21** |

Table 9 AHP general weight factor wishes

# Function analysis

To specify the function of the sorting method an analysis is made. The focus is on step 4 and 5. This function analysis can be changed during the designing process.

1. The fluid of the preservation pump comes into contact with the kidney that has just been removed from the donor’s body and is placed on the mechanism of the pump
2. This pump needs to be transported from the location of the donor to the location of the receiver of the organ
3. The fluid in the pump is stored in cold storage until the researcher has time to plate out the cells
4. The cells are plated onto gelatin-coated wells
5. The sorting process: the EC’s and the rest of the cells in the medium are sorted.
6. These EC’s are plated out mixed with medium

# Conclusion

The research concerning the renal endothelial cells has brought a new possibility of testing the HLA types. These EC’s need to be filtered out of a perfusion pump post-transplantation. Although the current method of filtration of EC’s out of the perfusion pump fluid is sufficient, this process takes a lot of time and is labor-intensive. Rosa Lammerts MD, working in the UMCG, has provided this problem in need of a solution.

The problem is in threefold; the plating of the beads is labor-intensive, time-consuming and the renal endothelial cells dedifferentiate into common endothelial cells. All of these parts contribute to the problem of slow progress for the research of the researcher.

In this problem analysis, enough inside is gained to proceed to the second step of the designing process, the synthesis phase. A new method should be found that efficiently sorts the cells out of the fluid but in a faster and less labor-intensive method. The most important requirements will be taken into account, as well as the wishes.

As a future perspective, also other cell types that are present in the fluid should be identified and the number of cells in this fluid counted. It is possible that these goals are inevitable to face in this designing process. The designing process can be read in the bachelor project following this thesis: “Isolation of renal endothelial cells out of a kidney preservation pump: a designing process.”

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