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Can Whole-heart Tissue Engineering Products by Recellularization from Scaffolds Be Utilized on Humans in the Next Ten Years?

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Abstract:

Diseases like organ failure lead to irreversible damage to our body, making organ transplantation the only treatment. However, though organ transplantation may be an effective treatment, problems of organ shortage and post-transplantation immune rejection still remain unresolved, making the development of alternative technologies vital. Organ regeneration is, therefore, a promising treatment as it aims to lower the chance of immune responses and may potentially solve the issue of organ shortage. An average procedure of organ regeneration has two steps: decellularization and recellularization. Decellularization techniques include physical, chemical, and enzymatic treatments, and these methods are often combined to maximize cell depletion, turning the organ into an extracellular matrix scaffold and minimizing the chances of immune rejection. Recellularization is the process of repopulating the scaffold with patient-specific cells, and is often completed through using perfusion systems by performing vascular perfusion and intramyocardial injections with the help of a bioreactor. This article explains the concept of whole-heart regeneration and assesses different methods of decellularization and recellularization to find the treatment with the highest efficacy and efficiency, while also conducting a case study on cultured epidermal autografts and primary research, in the form of a questionnaire, to look at the social acceptance of this technology, estimate the progress of whole-heart regeneration, and discuss its limitations.

Keywords: recellularization; decellularization; scaffolds; whole-organ recellularization; whole-heart recellularization; organ scaffolding.

1. Introduction

The rise of unhealthy lifestyle choices is undoubtedly causing great concern regarding the well-being of individuals. According to statistics, the proportion of men consuming over four units on their heaviest drinking days reached 37% in 2012 in England [1]. Consequently, problems begin to arise, resulting in irreversible changes to our bodies. Research shows 60% of factors related to one's health and quality of life are associated with lifestyle [2], one of which, frequently seen, is cardiovascular diseases. These issues may start off small but quickly become chronic, leading to severe consequences. Around 285 million people had cardiovascular diseases in 1990, and this rose to 430 million in 2010. Presently, this number is continuously rising, recently reaching 620 million [3]. In addition, severe cases of cardiovascular diseases can lead to death, for instance, heart failure, which leaves heart transplantation as the only treatment in the end stages. However, traditional heart transplantation requires a complex pre-transplantation procedure to be followed, including matching the donor and being waitlisted [4, 5]. All of these processes can cause delays and worsen the patient's condition, suggesting the need for heart recellularization as a potential solution.

Heart recellularization, or whole heart organ regeneration, is a newly developed technology where the organ undergoes decellularization to become a scaffold, followed by recellularization to become a newly produced organ. This technology's main goal is to reduce the chance of immune rejection of the transplanted heart, and when used effectively, it could decrease the risk of transplantation failure. Transplantation of recellularized hearts has immense potential to become an effective treatment for end-stage cardiovascular diseases.

The research conducted in this paper was driven by the potential to provide hope and treatment for patients who are suffering. The study will research organ regeneration, providing its concepts and potential procedures for the technology, discussing the advantages and disadvantages of several methods of decellularization, and elaborating on the potential methods of whole heart recellularization. A case study on cultured epidermal autografts was also performed to assist in concluding whether heart recellularization products from scaffolds can be utilized on humans in the next 10 years. Furthermore, primary research is conducted by sending a questionnaire to collect data on the level of social acceptance of this new technology,

which will also provide assistance for the conclusion stage of this study.

2. Methodology

In this dissertation, background research was conducted on organ regeneration, followed by an in-depth research and analysis on the procedure of how recellularized organs are produced. Papers about decellularization and scaffolding were also collected, along with a questionnaire designed to gather data on the social acceptance of recellularized organs or hearts, and to look for relationships among background information of the participants. This included, age, gender, and participants' attitudes towards recellularized hearts. In addition, an interview with professionals was planned. However, no responses were received from the contacted professionals in time due to their availability.

2.1 Literature Research

In this study, peer-reviewed papers and scientific studies were used in the literature research. During the research, databases including PubMed, the library of the University of Hull and Google Scholar were used. Examples of the keywords used in research include: 'whole heart organ recellularization', 'whole heart engineering', 'cardiac tissue engineering', 'whole heart organ decellularization', 'organ scaffolding' and 'whole heart bioengineering'. This search strategy ensures a thorough study on a large quantity of collected literature to screen and evaluate. Additionally, the reference lists of collected papers were also used to find other relevant papers for thoroughness, as some related papers may not fully match the keywords used in the original search.

2.1.1 Analysis of Literature

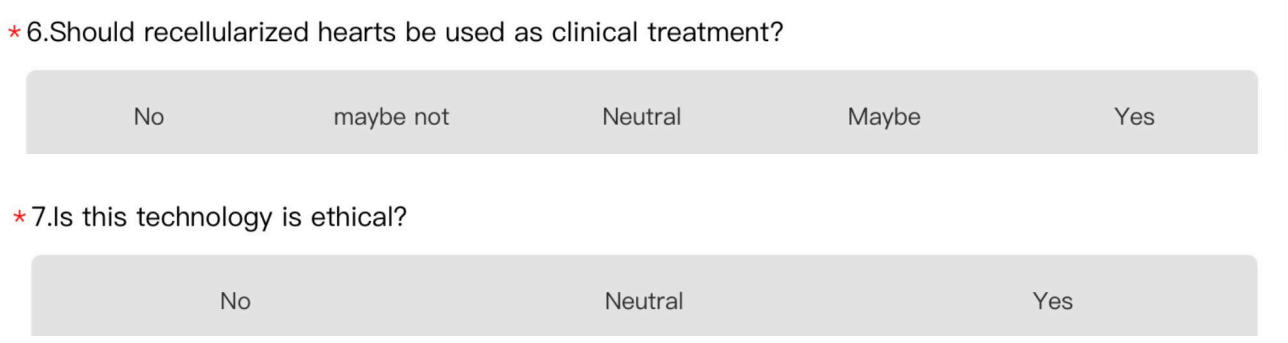
During the research, a large number of literatures were found. However, some were found to be unsuitable for use upon analysis. The screening criteria for literature in this study consider four factors: currency, accuracy, authority, and relevance. Collected literature is screened based on the title, abstract, and contents respectively. The currency of each paper was analysed by checking how recently the paper was published, ensuring the results and conclusions are up to date. To be considered suitable, the paper must be within a 5 to 10-year period since its publication. However, exceptions are made if the paper has high research value. The accuracy and authority of the papers were also analysed by checking the citations and whether the paper belonged to a top-ranked journal, as papers published in such journals are generally peer-reviewed for accuracy and authority. The relevance of the papers was analysed by carefully reading through the contents, as this enables a thorough understanding of each paper and allows conclusions to be made about whether the paper is relevant and suitable for the study.

Primary Research

Questionnaire Design

The designed questionnaire consists of nine questions in total and includes two types of questions: Likert scale questions and multiple-choice questions. In the questionnaire, questions 1-3 were multiple-choice, collecting data on the participants' basic personal information, such as gender, age groups, and education level. Questions 4-5 were designed to determine whether the depth of knowledge on recellularized organs would influence responses to questions 6-9.

Questions 6-9 ask about the ethicality, utilization, and acceptance by law of recellularized organs and hearts (Figure 1). These questions are more critical and are based on personal opinions. This is the most important part of this questionnaire, as they focus on social acceptance. In addition, at the beginning of the questionnaire, a brief introduction to recellularized organs is provided, alongside a diagram to better illustrate the concepts. This helps the participants to have a basic understanding of organ recellularization before answering the questions and assists them in making more informed decisions. Furthermore, the introduction also mentions that the study will keep their responses confidential to encourage the participants to give an honest response based on their individual thoughts.



*8.In emergency situations, should recellularized organs should be used as treatment?

No	Maybe not	Neutral	Maybe	Yes

*9.Should this technology be accepted by law?

No	Maybe not	Neutral	Maybe	Yes

Figure 1: critical questions, questions 6-9

2.1.2 Sampling Method and Data Analysis

This questionnaire uses volunteer sampling and was distributed online via social media platforms and Questionnaire Star. Overall, 371 samples were collected. Then, invalid responses were excluded to enhance the reliability of the results, leaving 323 remaining samples. Invalid responses needed to be deleted as these participants spent only 30 seconds or less when answering the questionnaire, indicating it is unlikely that they had read through the introduction and questions carefully, making their responses invalid. Deleting these invalid responses improves the data quality and contributes to the investigation of accurate relationships, as the reliability of the responses is ensured. After data cleaning, analysis was performed to find relationships between social acceptance and characteristics of the participants.

3. Results and Discussion

3.1 Literature Research

3.1.1 Whole Organ Regeneration

Organ regeneration is a developing technology that has the ability to produce new organs from the scaffolds of existing organs for transplantation use. These organs, when fully developed, have the potential to solve a range of problems in the field of organ transplantation. A good example of such problems is the long waiting time on the waitlist to find a matching donor.

The general procedure for organ regeneration has two steps: organ decellularization and organ recellularization (Figure 2). During organ regeneration, the native organ must first go through decellularization, which depletes cells from the organ and removes cellular components, including DNA. After this, recellularization is performed with patient-specific cells, thus reducing the rates of immune rejection and the chances of failure during transplantation. Because recellularization is performed on the decellularized extracellular matrix scaffold, when recellularization occurs, cellular migration, proliferation, and differentiation will all be regulated [7]. Once both procedures are completed, organ regeneration is accomplished.

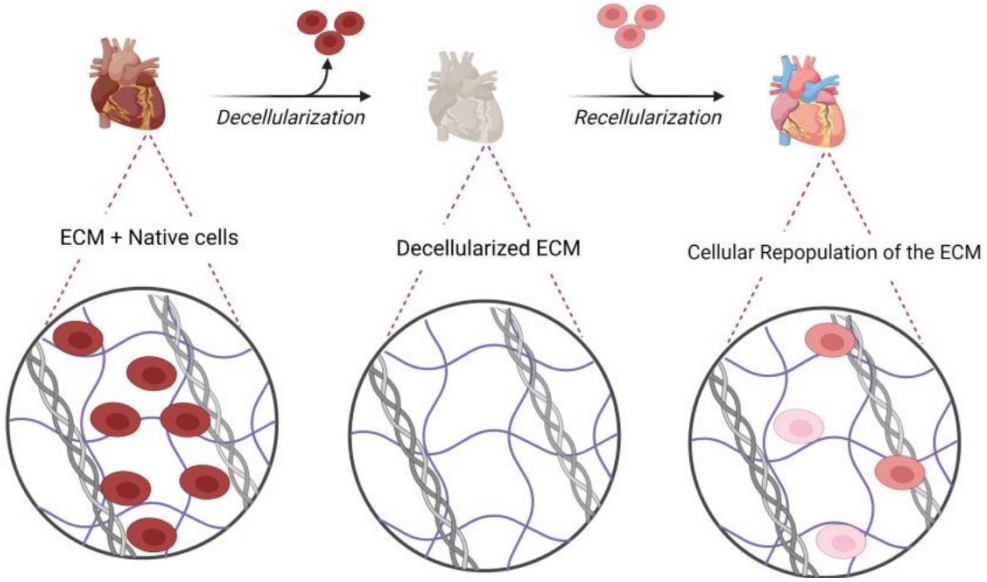


Figure 2: illustration of the procedure of whole heart regeneration [8].

3.1.2 Decellularization

Organ decellularization is the first step of organ regeneration, where the native cells of an existing organ are depleted to turn the target organ into a structural extracellular matrix scaffold, biomaterials created by using human or animal organs/tissues to undergo decellularization to remove immunogenic cellular components [9], as a template for recellularization of the organ. This process is a complicated procedure involving the removal of existing cellular components without damaging the ultrastructure of the natural organ. However, it can be achieved through the combined use of multiple methods of decellularization, including physical, chemical, and enzymatic treatments, maximizing the advantages of decellularization [10]. The use of multiple techniques enables the efficiency of organ decellularization to be at its optimal level, maximizing the depletion of cells while keeping the ultrastructure and proteins of the organ undamaged. One example of such methods uses a combination of physical and chemical treatment, by using the freeze-thaw cycle to repetitively freeze-dry the organ, thawing it in a buffer solution, and using Triton X-100 with a hypotonic solution [9].

3.1.2.1. Physical Treatment

Physical treatments manipulate physical aspects of the organ, such as the freeze-thaw cycle, immersion, agitation, and perfusion. These methods often achieve high efficiency in decellularization but may damage the organ scaffold [9].

3.1.2.2. Chemical Treatment

Chemical treatments involve using chemicals to break cellular bonds and to eliminate cellular contents. Examples of these treatments include using ionic and non-ionic detergents, acids, bases, and hypotonic or hypertonic solutions [11]. However, these methods often lack the ability to remove DNA and will also damage the ultrastructure of the organ [9]. Sometimes physical and chemical methods are combined to minimize their disadvantages.

3.1.2.3. Enzymatic Treatment

Enzymatic treatment can often be used as a pre- or post-detergent decellularization method to maximize the effectiveness of decellularization. These methods involve using enzymes to catalyze reactions that break down the cellular components. An example of this would be the use of nuclease to break down and remove DNA contents [12].

3.1.3 Decellularization of Human Heart

The heart muscle is a vital component of our circulatory system, which primarily acts as a pumping device for our blood, supplying nutrition and oxygen to other organs. Thus, the heart requires great strength and endurance, which results in its ultrastructure being composed of types 1, 2, and 3 collagens, fibrillin, hyaluronan, laminin, fibronectin, and proteoglycans (Figure 2) [11]. For cardiovascular decellularization to be considered successful, the heart's native characteristics and architecture must be kept unchanged, allowing it to retain its original functions. This facilitates differentiation, specific to the heart, and assists in the regulation of cell adhesion, growth, and differentiation, underscoring the importance of retaining the ultrastructure of the heart. This needs to be considered when picking a suitable decellularization treatment.

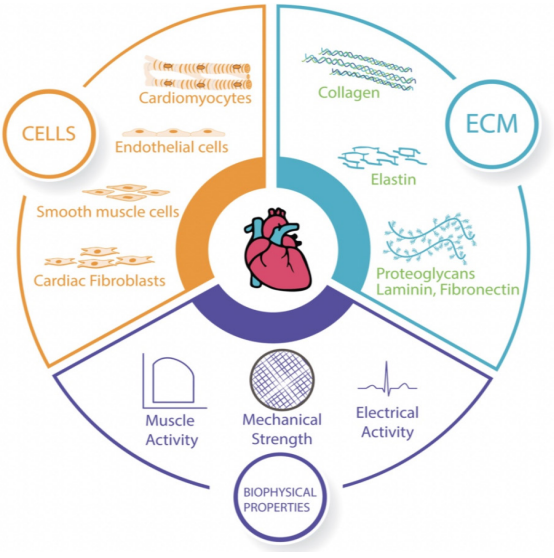


Figure 3: picture providing basic information about whole heart regeneration, showing the vital components [13].

3.1.3.1. Methods of Human Heart Decellularization

A successfully decellularized heart will need to retain the organ scaffold, as the native ultrastructure plays a vital role in organ regeneration, aiding future developments of the organ and its functioning [9]. Thus, protecting the ultrastructure is crucial in whole heart decellularization. However, the main goal of organ regeneration is to create a transplantable organ with reduced rates of immune rejection by removing cellular contents. Therefore, the ability to remove cellular components is also an essential factor of decellularization, and methods of decellularization must achieve both factors to a certain extent.

Recently, common techniques of whole heart decellularization involve a combination of detergents and decellularizing agents, including pre-decellularization processes [9]. Research suggests that the use of chemical treatments is an effective method of whole heart decellularization [12]. These treatments function by breaking covalent bonds to remove DNA contents to achieve cell removal. An example is the combined use of SDS detergent (sodium dodecyl sulfate), an ionic detergent, and Triton X-100 as a detergent-based method of chemical decellularization by perfusion or rinsing. Data indicate that using 0.5% SDS followed by 1% Triton X-100 treatment is an effective method of decellularization. This is because SDS detergent has a strong ability to remove cells, with experimental data showing that a 6-hour exposure to 0.5 percent SDS detergent results in 98 percent DNA removal. This far exceeds other chemical treatments, such as using Triton X-100 alone, which results in incomplete decellularization [12]. However, due to its strong ability, SDS detergent can damage the organ scaffold. Nonetheless, a low concentration of SDS combined with Triton X-100 enables thorough cell removal while preserving the heart's ultrastructure [14]. Additionally, physical and enzymatic treatments, such as the freeze-thaw cycle, can be used as pre-detergent-based or post-detergent-based decellularization treatments to further enhance results. This combined treatment may be a promising method of decellularization during whole heart regeneration.

3.1.4 Recellularization

Recellularization is the second step of organ regeneration. This process is performed on the decellularized organ scaffold by repopulating it with recipient-specific stem/progenitor cells to eventually create a transplantable patient-specific organ [15]. This process requires a range of cells for cell repopulation due to the complexity of the organ. In the same organ, different cells are needed to repopulate different areas.

3.1.4.1. Recellularization and Bioreactor

Due to its complexity, the heart requires a supply of cardiomyocytes for cellular repopulation of the cardiac muscles and also requires endothelial cells for reendothelialization [15, 16]. A source of cardiomyocytes is vital to whole heart recellularization due to their high abundance in the composition of a human heart, comprising roughly 30 percent of the heart [16]. Additionally, endothelial cells, smooth muscle cells, perivascular cells, and fibroblasts are also essential to the heart's composition and are all required for cell repopulation [17].

In a general process of whole heart recellularization, the use of a bioreactor is essential to provide a stable environment for cell proliferation after cell seeding. The bioreactor is widely used in organ recellularization as it mimics the in vivo environment and conditions of the body, providing the organ with vital needs such as oxygen, while also monitoring factors like pH level [13]. This device allows cell repopulation to take place at an optimal level, enabling cell proliferation to occur. All methods for organ recellularization should consider these factors and find ways of incorporating bioreactors and cells to achieve maximal results.

3.1.4.2. Methods of Human Heart Recellularization

Recellularization of a human heart requires large amounts of highly specialized cells, which indicates that these cells do not proliferate like other cells. Due to this, for repopulation of the human heart to occur normally, a large number of human cardioblasts are needed, suggesting the need for a method to induce progenitor cells, which can differentiate into specialized cell types, including human cardioblasts. Research shows that human embryonic stem cell-derived cardiomyocytes can efficiently engraft onto the organ scaffold, maintaining the heart's native characteristics [18]. These cells are a promising choice for recellularization.

After this, cells may be seeded onto the organ scaffold. For the human heart, cell repopulation may be performed by using a cell perfusion system, which includes the use of intramyocardial injections and perfusion of the vascular systems [19].

Once cell seeding is performed, a bioreactor is used to maintain a stable environment for regrowing the heart. The bioreactor mimics the in vivo conditions of the body by maintaining the pH and temperature at optimal levels for cell repopulation. Additionally, the bioreactor provides nutrition and oxygen for cell growth. This information provides a general overview of organ recellularization, suggesting possible methods and uses of cells.

3.1.5 Development of Whole Heart Recellularization

3.1.5.1. Pre-clinical Trial Development

The application of a newly developed treatment requires several stages. First, after the discovery of the treatment, it

needs to go through the development phase, where a series of experiments are conducted to examine the performance of the treatment. This is where information like side effects is gathered [20]. After the developmental phase, preclinical research is performed to ensure the treatment does not cause serious harm to the patient. Typically, preclinical research is performed on animals to observe the effects of the treatment, and detailed information on toxicity must be provided [20]. Once preclinical research is completed, clinical trials may be conducted. Clinical trials are designed to answer targeted research questions about the developing technology and to test the treatment on humans to determine its efficacy.

3.1.5.2. Stages of Clinical Trials

Clinical trials ensure the efficacy and safety of a treatment and require volunteers suffering from a certain condition to participate. This gives patients an opportunity to receive treatment with newly developed technology, potentially saving lives. A general overview of clinical research includes four stages. Phase 1 clinical trials involve 20 to 100 participants; Phase 2 involves several hundred; Phase 3 involves 300 to 3,000; and Phase 4 involves several thousand participants [21]. Furthermore, single case research may also be conducted before phase 1 clinical trials. These studies apply the treatment to single participants to further guarantee the safety of the treatment.

3.1.5.3. Case Study of CEA

CEA, or cultured epidermal autografts, is a type of biotechnology used to treat skin damage. This treatment uses stem cells derived from skin to cultivate biomaterials that are used to cover wounds to promote healing [22]. CEA is similar to organ recellularization as it is also a form of biotechnology using stem cells as part of therapeutic treatment and involves transplantation. Both CEA and organ recellularization are types of biotechnology that use patient-specific stem cells to inhibit immune rejection, with the completed product transplanted to the patient. These two treatments have closely related concepts, which is why CEA is chosen as the case study for this work.

Based on research, the first clinical trials of CEA were conducted in 1980, and during the 1980s and 1990s, clinical research moved from single-case studies to Phase 1 clinical trials (figure3). In the early 2000s, Phase 2 clinical trials were conducted, and after 2005, Phase 3 and Phase 4 trials were conducted [22]. The development of CEA from single-case studies to large end-stage phase 4 clinical trials took approximately 24 years, from 1981 to 2005. Based on the similarities between CEA and organ regeneration, it is likely that whole heart regeneration may follow a similar pattern in its development. However, whole heart engineering is more complicated because of the heart's complex vasculature and vital properties that require effort to maintain, such as strength and endurance. Compared to CEA, whole heart engineering requires more research in preclinical trials, as the effect of failure may be more extensive due to its complexity. Because of these factors, whole heart recellularization may require more time before it can be utilized on humans. For CEA, the first publication appeared in 1975, and clinical research began ten years later [23]. For whole heart engineering, the first technique was introduced in 2008 [24]. Now, sixteen years later, preclinical research is being conducted. Although the technology is not perfected, recellularized hearts are closer to clinical research.

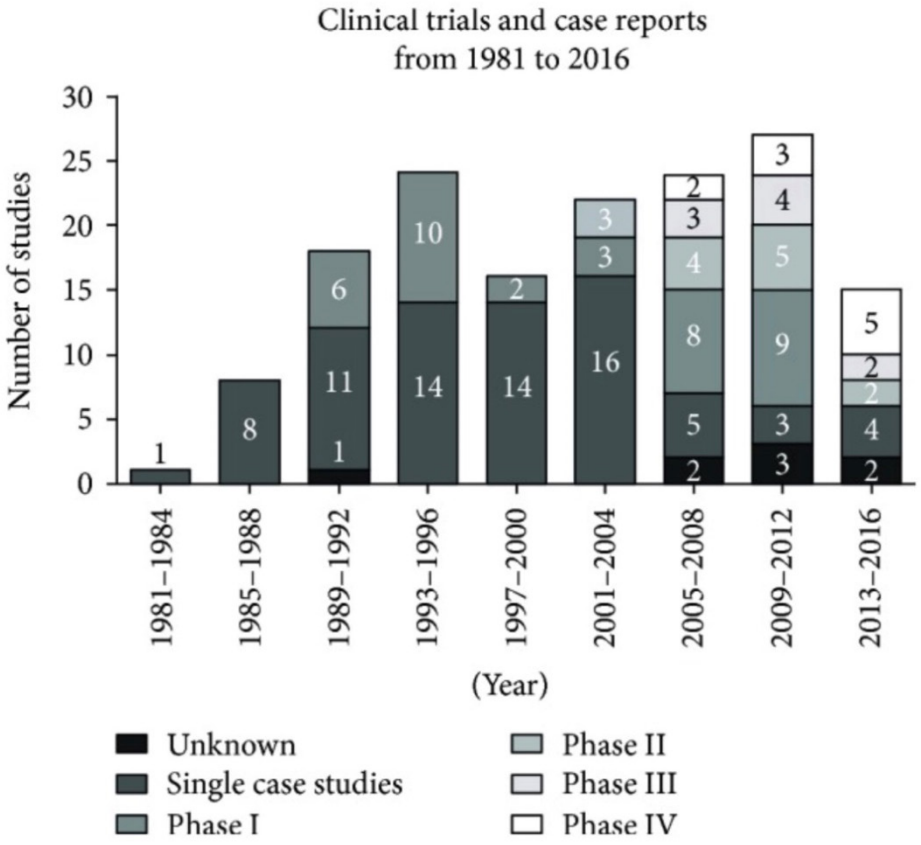


Figure 4: Graph demonstrating case studies of CEA performed from 1981 to 2016 [22].

3.1.5.4. Limitations in Organ Regeneration

Although significant accomplishments have been made in the field of organ regeneration, there are still many aspects requiring further research, and many problems still faced during the developmental process. One of these is finding a way of decellularization that keeps the organ-specific ultrastructure undamaged while effectively removing cellular components. However, this problem can be resolved by researching a range of methods of decellularization and applying combinations of existing methods. Manipulation of these decellularization methods provides a good method for both the removal of cellular components and the preservation of the scaffold. Another potential problem is the creation of a bioreactor that efficiently maintains the environment required for the growth of cardiac and other differentiated cells around the decellularized ECM scaffold [13]. Furthermore, the long-term viability of recellularized organs is not yet known. Because of the large use of different chemicals in the process of constructing these organs, their effects on the organ and its long-term functionality are yet to be determined. This demonstrates the importance of clinical research in identifying these effects.

3.2 Primary Research

3.2.1 Questionnaire Results

3.2.1.1. Background Information of Participants

In the questionnaire demonstrated in Figure 5, a total of 371 responses were collected and 323 valid responses were used for data analysis. Of the 323 respondents, 43% were female participants, leaving around 57% male participants. For the educational level of the participants, this questionnaire mainly collected information from participants with a bachelor's degree. Most of these respondents do not work in fields related to biomedicine or have a relevant major. In addition, participants aged from under 18 to above 80 were included, with the majority being in the 30-39 age group and the 18-24 age group, which together comprised more than half of the total participants.

Questions asked:

a) Please select your gender

b) Is your career related to biomedicine?

c) Have you ever heard of organ recellularization before this?

d) Please select your age group

e) What is your educational level?

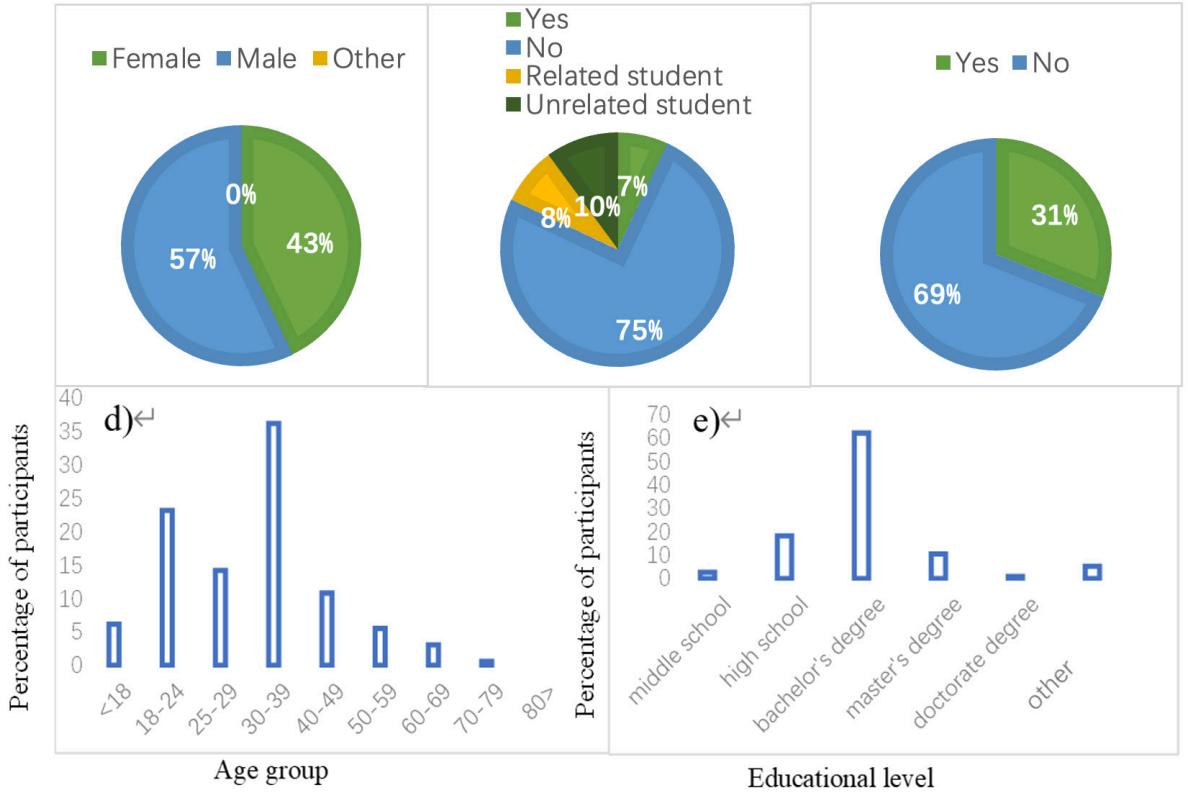


Figure 5: Background Information of Participants

3.2.1.2. Social Acceptance of Whole Heart Recellularization

Overall, the questionnaire results indicate positive social acceptance. The graphs show a general trend of positive feedback on recellularized hearts, with 58% of participants choosing a positive response for all critical questions. According to Figure 6, the majority of participants were either neutral (35%) or had a positive attitude (58%) toward recellularized hearts as a clinical treatment. For all critical questions, more than half of the participants chose 'yes', 'maybe', or 'neutral' (93%).

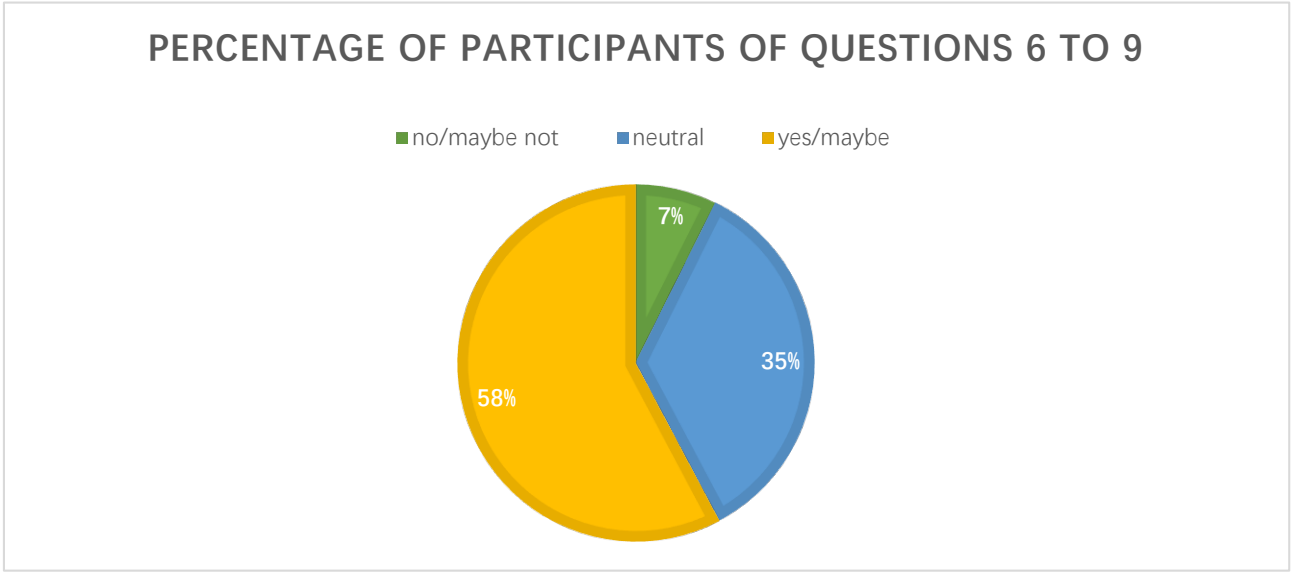


Figure 6: Percentage of participants for each choice of Questions 6 to 9

In addition, as observed in Figure 7, when participants were not given the choice of 'maybe', they tended to choose neutral instead (66%). This shows that some participants were uncertain about giving a positive answer and instead chose 'maybe'. It also implies they were less keen to giving an affirmative yes (31%) or a no (3%).

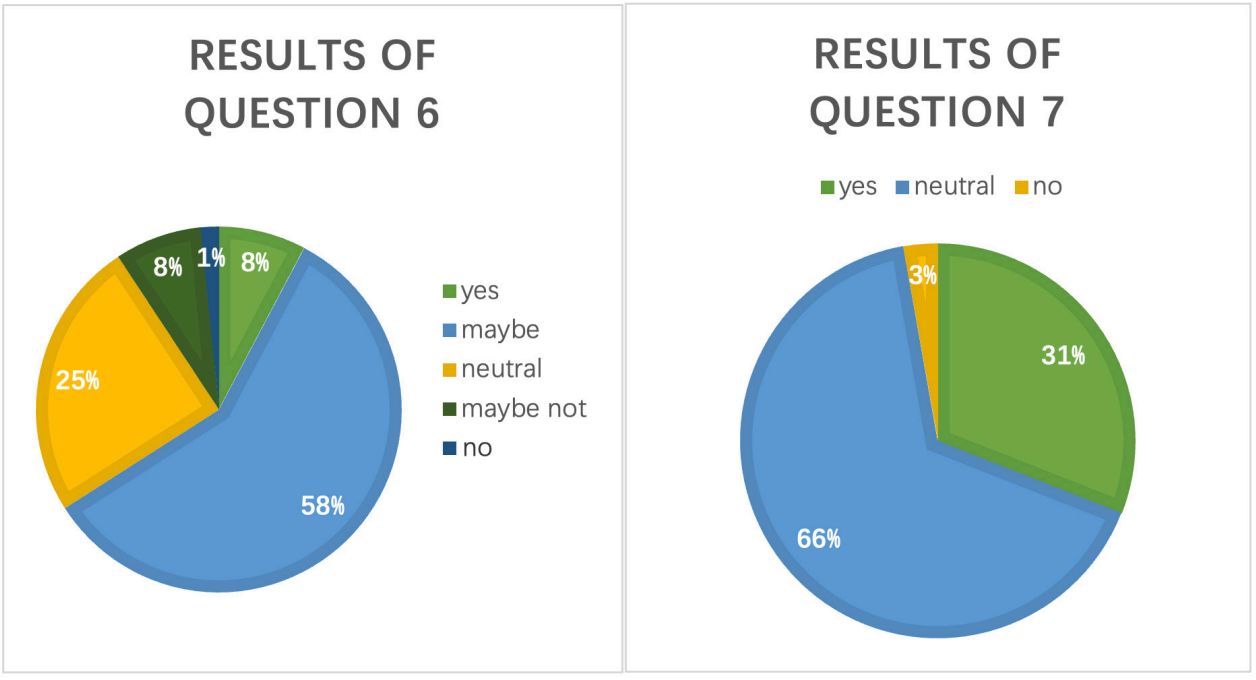


Figure 7: Graph of results of questions 6 and 7

Generally, there seem to be no difference between gender and social acceptance of this technology. According to figure 8, both male and female participants had similar percentages for both positive (female 92% and male 90%) and negative choices (female 8% and male 10%).

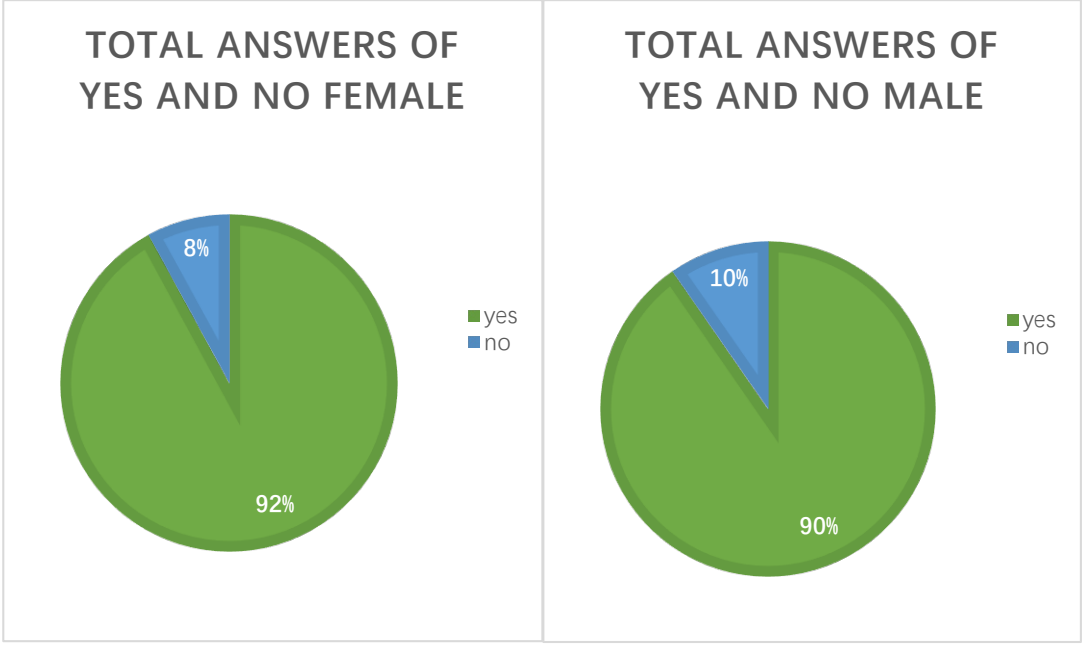


Figure 8: Proportions of 'yes' and 'no' responses for male and female participants.

Next, results of question 5 were analyzed based on age. The percentages of participants were compared between

those who had heard of organ recellularization before and who had not. The participants were split into two groups: above 50 and below 50, to find any relationships between age and likelihood of knowing organ recellularization. The following graphs show the analyzed results.

Question 5: Have you ever heard of organ recellularization before this?

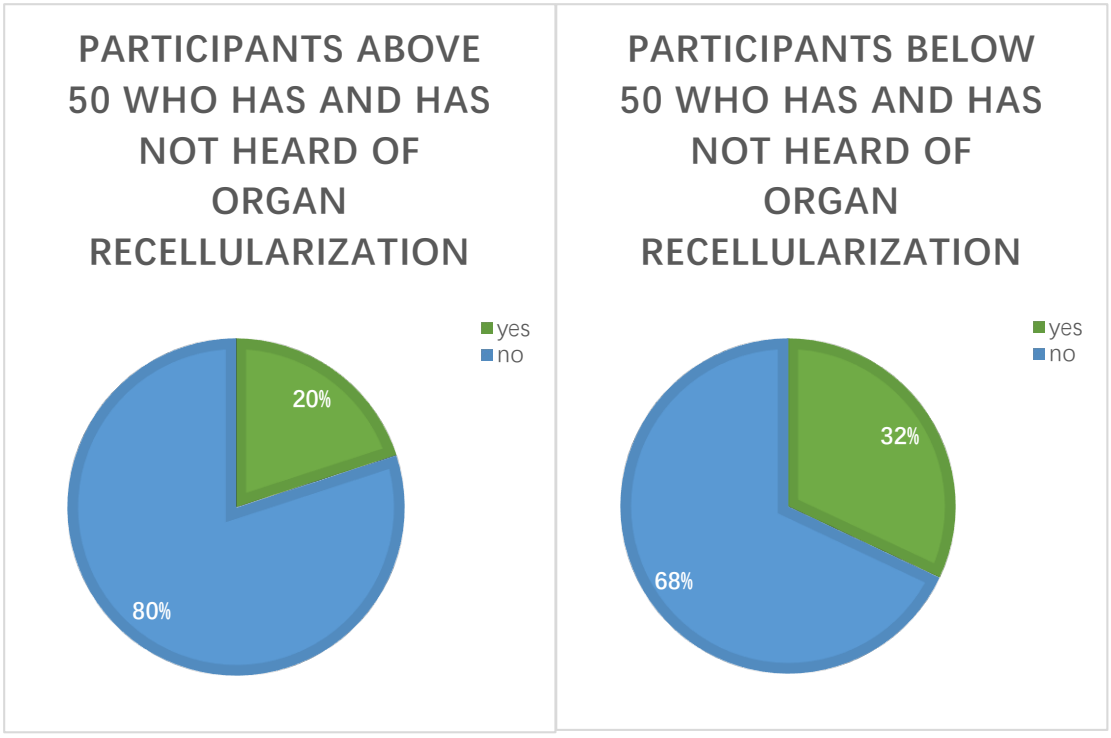


Figure 9: Graphs of participants above, and below 50 and whether they have heard of organ recellularization.

Evidently, for the first group of participants aged above 50, only 20% had heard of organ recellularization before, whereas for participants below 50, this increased to 32%, showing a relationship between age and the possibility of knowing about organ recellularization. Based on the data, younger people are more likely to know about organ recellularization (32%). This difference may be because elderly people are inclined to use electronic devices less frequently, so they are less likely to hear about modern advances in medical treatments.

According to Figures 10 and 11, participants who have heard of organ recellularization before seem less likely to choose 'no' or 'maybe not'. Participants who picked 'no' or 'maybe not' who had heard of organ recellularization before for Questions 8 and 9 account for 37% and 27%, respectively, while participants who have not heard of organ recellularization before account for 63% and 73% of the number of participants who chose negative options.

Question 8: In emergency situations should recellularized organs be used as treatment?

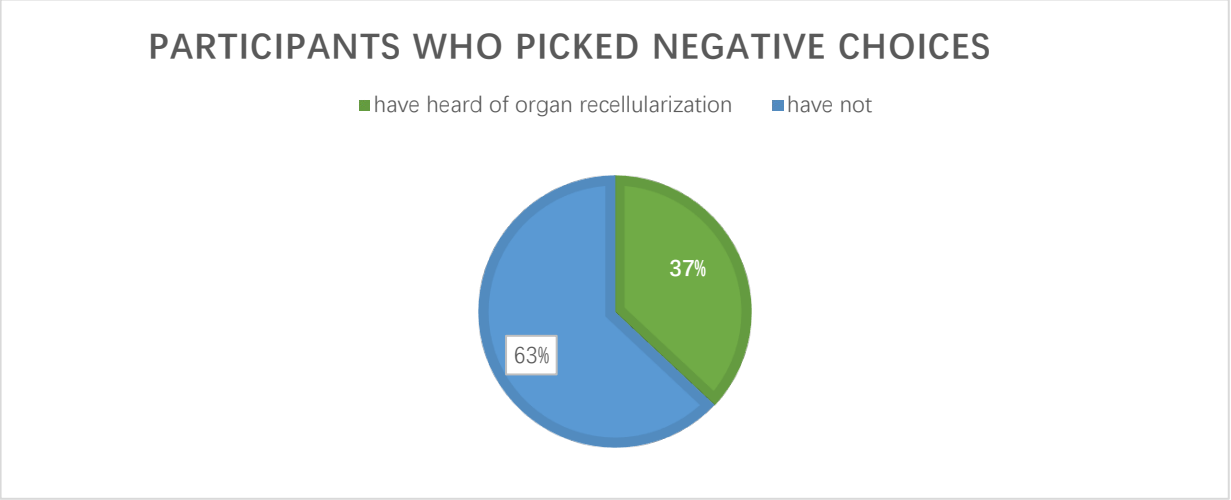


Figure 10: Graph of results of question 8

Question 9: Should this technology be accepted by law?

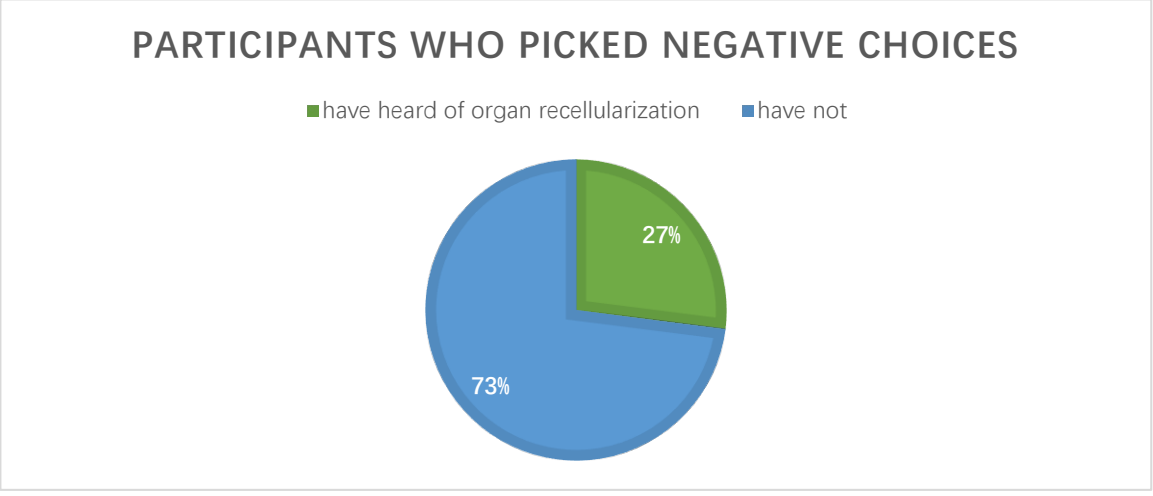


Figure 11: Graph of results of question 9

3.2.1.3. Improvement in Social Acceptance

From the collected data, a generally positive attitude towards organ recellularization and the usage of recellularized hearts can be observed. However, there is a majority who are neutral or uncertain about picking 'yes', suggesting there are still areas for improvement in terms of social acceptance. One way to enhance social acceptance of this technology is to spread awareness about its purpose and advantages. The conducted questionnaire also shows that the more information participants know about organ recellularization, the more positive their attitudes are toward this technology, which suggests that if awareness of this technology is spread more effectively, more people would gain knowledge about organ recellularization, and an increase in social acceptance may be seen. For some individuals, resistance to organ recellularization may be entirely due to misunderstanding or lack of understanding of this technology. Dissemination of information and knowledge about organ recellularization is a better way to increase social acceptance.

4. Evaluation

Firstly, one limitation of this study is the primary research. In the primary research, the questionnaire was distributed using volunteer sampling, which means the generalizability is limited. In addition, this study mostly considered participants in China, suggesting limited generalizability of the results due to geographic limitations. However, a large sample of 323 participants with a relatively distributed gender ratio of 1:1.3 was collected, meaning the data is not only limited to a specific gender, preventing potential bias in gender, which is a strength of this study. Furthermore, participants from a wide range of age groups were

included to ensure the accuracy of the results.

Secondly, there were also limitations in the literature research. During the literature research, a case study was performed on cultured epidermal autografts to determine the developmental stage of recellularized hearts for potential use in humans. Though these technologies have their similarities, there are also differences. One difference is the purposes of these technologies. The purpose of heart recellularization is to produce hearts with the recipient's own cells to lower the chances of immune rejection after implantation and to reduce the demand for transplantation, whereas for CEA, the main purpose is to promote healing of wounds in the skin [22]. This difference may lower the validity of this case study. However, this study still has its research value, as both studies involve the use of stem cells to lower immune responses, showing the potential similarities in the developmental process of these technologies. The case study of CEA could provide a potential route for heart recellularization and its future utilization, making this a strength of this study. In addition, this study also performed literature research with analyzed literature, each checked for authority, accuracy, recency, and relevance to this research. This ensures that each piece of literature is reliable and useful, increasing the validity of this research and providing this study with value and reliability.

5. Conclusion

Through this research, whole-heart engineering seems to be mostly accepted by society, with positive feedback from the conducted questionnaire. From the results of the conducted primary research, a relationship between how much people know about heart recellularization and their social acceptance was observed. When participants have a deeper knowledge of heart recellularization, they are less likely to choose negative options for social acceptance, which shows that spreading awareness of this technology can increase acceptance in society. If more knowledge about this technology is taught to the public, social acceptance of this treatment has the potential to reach new heights. This concludes that social factors will likely have a positive impact on the utilization of recellularized hearts when the majority has a supportive attitude.

Regarding technological progress, whole-heart engineering is still developing. An example is the desired perfection of a bioreactor that can maintain the ideal environment for cell proliferation of the heart during recellularization [13]. However, an overview of whole-heart engineering, including decellularization and recellularization, is already present. From research, a potential method of whole heart regeneration would be using 0.5 % SDS detergent as a decellularization method. In addition, the freeze-thaw cycle may be used to further assist cell depletion. Next, a cell perfusion system using intramyocardial injections and perfusion of the vascular systems may be used as a method of recellularization, while also needing a bioreactor to maintain an optimal environment to induce cell proliferation.

Heart recellularization is becoming a promising approach for solving the problem of organ shortages. The technology has already entered preclinical trials testing the recellularized heart in animals. Research shows that CEA took 10 years from its first publication in 1975 to reach the stage of clinical trials [23]. Recellularized hearts may follow a similar path. Whole heart engineering was first developed in 2008 [24]. Thereafter, this technology has evolved dramatically, becoming extremely close to clinical research. Within ten years, it is likely that the utilization of recellularized hearts will be tested on humans, but only in single-case or Phase 1 clinical trials, which should involve 20 to 100 diseased individuals [21]. From a current perspective, the utilization of recellularized hearts in humans is probable, whereas mass utilization is difficult to achieve due to its cost and complexity. However, considering the power of scientific developments, recellularized hearts have the potential to become a common treatment for end-stage cardiovascular diseases. With constantly evolving technology, more research may be conducted in this field, perfecting this technology, lowering its cost, and creating a modernized treatment that saves lives.

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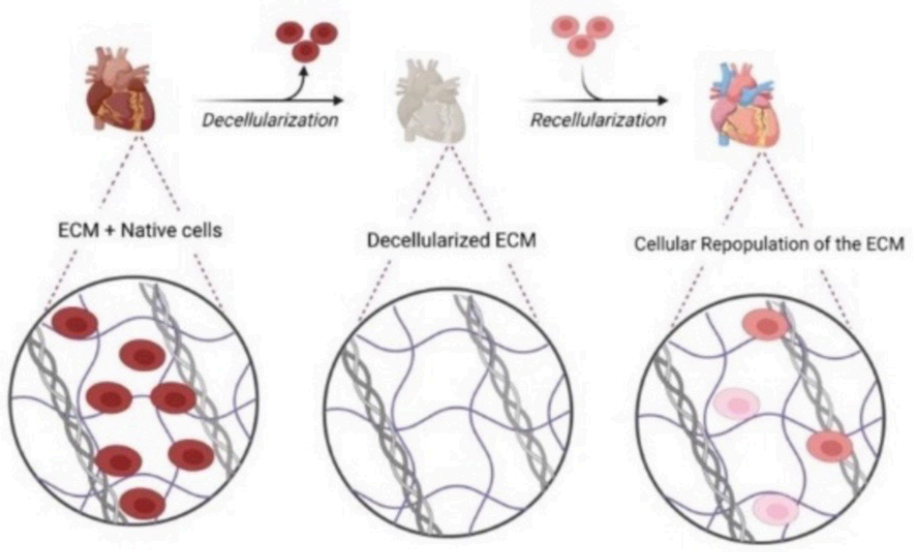
7. Appendix

7.1 Questionnaire

Please read the following information before completing the questionnaire.

Recellularized organs are organs which have undergone decellularization, meaning to have native cells of the organ removed, typically by perfusion of chemicals, thus, turning the organ into a scaffold. After repopulating the scaffold with stem cells, a ***recellularized organ*** is produced. This new technology is still in it's developing process, but transplantation of such organs has been successfully completed for simple structures like the skin. The aim for these organs is to ***lower or inhibit potential immune rejection*** by depleting native cells of the organ, to be transplanted, and repopulating it with cells from the patient. Furthermore, the next step of this technology would be making adjustments on small details that would have a large impact on the final product. Perfection in this technology would have a large improvement on the lack of human organs for transplantation.



Source of this photo: https://doi.org/10.3390/bioengineering10010106

This questionnaire is for research purposes only and your response will not be shared to anyone except the investigator. ***Your information will be entirely kept confidential***. Please answer the following 9 questions ***based on your own opinion and status***.

* 1.Please select your gender

- ○ Male
- ○ Female
- ○ Other_____

* 2.Please select your age group

- ○ < 18
- ○ 18–24
- ○ 25–29
- ○ 30–39
- ○ 40–49
- ○ 50–59
- ○ 60–69
- ○ 70–79
- ○ 80 ⩾

* 3.What is your current educational level?

- ○ middle school
- ○ High school
- ○ Bachelor's degree or equivalent
- ○ Master's degree or equivalent
- ○ Doctorate degree or equivalent
- ○ Other_____

* 4.Is your career related to biomedicine?

- ○ Yes
- ○ No
- ○ No, I am a student but my subjects are linked to it
- ○ No I am a student and my subjects are not linked to it

* 5.Have you ever heard of organ recellularization before this?

- ○ Yes
- ○ No

* 6.Should recellularized hearts be used as clinical treatment?

No	maybe not	Neutral	Maybe	Yes
.				

* 7.Is this technology is ethical?

No	Neutral	Yes
.		

* 8.In emergency situations, should recellularized organs should be used as treatment?

No	Maybe not	Neutral	Maybe	Yes
.				

* 9.Should this technology be accepted by law?

No	Maybe not	Neutral	Maybe	Yes
.				

7.2 Questionnaire Results

Question 1: Please select your gender

Option	Number of people	Proportion
Male	183	56.66%
Female	139	43.03%
Other	1	0.31%
Number of valid respondents	323	

Question 2: Please select your age group

Option	Number of people	Proportion
< 18	20	6.19%
18-24	75	23.22%
25-29	46	14.24%
30-39	117	36.22%
40-49	35	10.84%
50-59	18	5.57%
60-69	10	3.1%
70-79	2	0.62%
80 ≥	0	0%
Number of valid respondents	323	

Question 3: What is your current educational level?

Option	Number of People	Proportion
middle school	9	2.79%
High school	59	18.27%
Bachelor’s degree or equivalent	201	62.23%
Master’s degree or equivalent	34	10.53%
Doctorate degree or equivalent	3	0.93%
Other	17	5.26%
Number of valid respondents	323	

Question 4: Is your career related to biomedicine?

Option	Number of People	Proportion
Yes	22	6.81%
No	243	75.23%
No, I am a student but my subjects are linked to it	26	8.05%
No I am a student and my subjects are not linked to it	32	9.91%
Number of valid respondents	323	

Question 5: Have you ever heard of organ recellularization before this?

Option	Number of People	Proportion
Yes	100	30.96%
No	223	69.04%
Number of valid respondents	323	

Question 6: Should recellularized hearts be used as clinical treatment?

Option	No	maybe not	Neutral	Maybe	Yes
Proportion	5(1.55%)	25(7.74%)	80(24.77%)	188(58.2%)	25(7.74%)

Question 7: Is this technology ethical?

Option	No	Neutral	Yes
Proportion	9(2.79%)	214(66.25%)	100(30.96%)

Question 8: In emergency situations, should recellularized organs should be used as treatment?

Option	No	Maybe not	Neutral	Maybe	Yes
Proportion	7(2.17%)	23(7.12%)	65(20.12%)	143(44.27%)	85(26.32%)

Question 9: Should this technology be accepted by law?

Option	No	Maybe not	Neutral	Maybe	Yes
Proportion	9(2.79%)	17(5.26%)	92(28.48%)	133(41.18%)	72(22.29%)

7.3 Graphical Results for Critical Questions

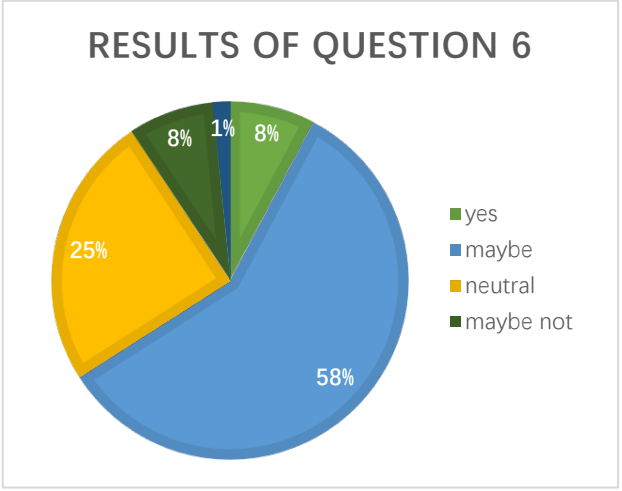


Figure 12: Graph of results of question 6
Should recellularized hearts be used as clinical treatment?

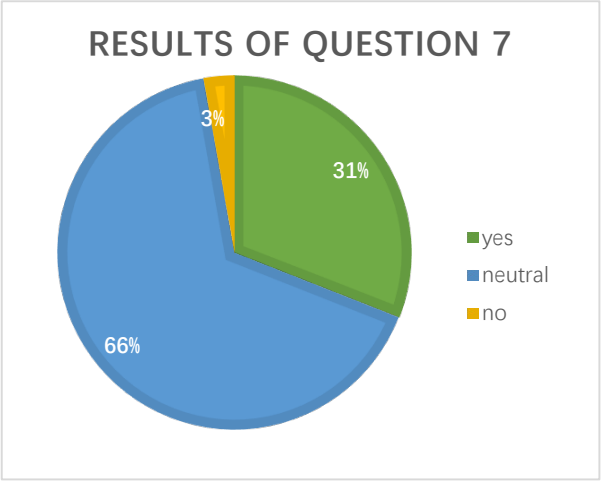


Figure 13: Graph of results of Question 7
Is this technology ethical?

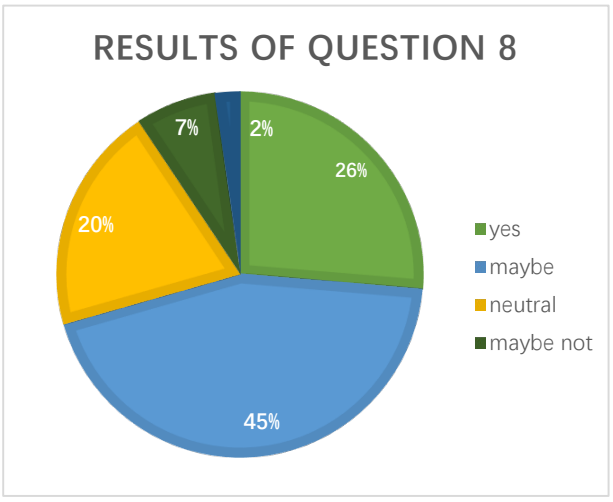


Figure 14: Graph of results of Question 8

In emergency situations, should recellularized organs be used as treatment?

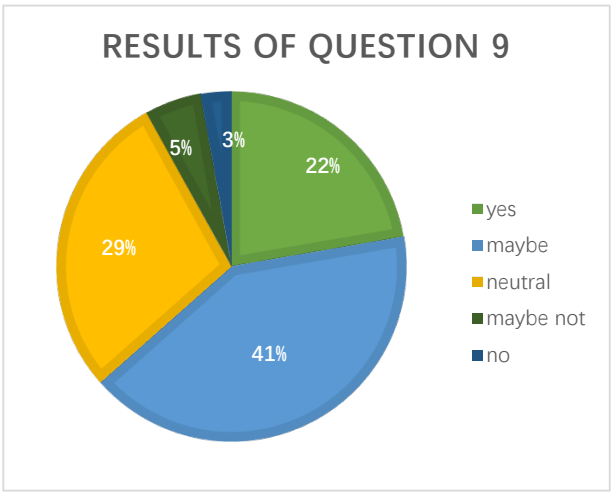


Figure 15: Graph of results of Question 9

Should this technology be accepted by law?