Experimental and Bioengineering Research 2022

Novel bioengineering technique to obtain kidney scaffolds *in vivo*

Sophie Oms[1], Andrea Flores Abarca[1], Ivanette Cubero Munoz[1], Marta Garcia Diez*[1], and Pedro M. Baptista*[1]

[1]Biomedical Engineering Laboratories, Universidad Carlos III, Betancourt Building, 1.0.G08, Av. Universidad 30, 28911 LEGANES (MADRID) SPAIN.

December 16, 2025

Abstract

Over 105,000 people are currently on the national waiting list in the United States of America, alone. As of February 2022, it has been shown that 83% of the people on this list are waiting for a kidney [1]. The creation of a transplantable graft to permanently replace kidney function would address donor organ shortage and the morbidity associated with immunosuppression. Such a bioengineered graft must have the kidney's architecture and function, and permit perfusion, filtration, secretion, absorption, and drainage of urine [2].

A novel technique to bioengineer a kidney in only three major steps was created.

(**Figure 1**). We harvested bone marrow from a rat to obtain its Mesenchymal Stem Cells (BM-MSC). Afterwards, the same rat's kidney (with its pertaining vasculature and ureter) was decellularized by washing it with deionized water. Finally, the acellular kidney scaffold was recellularized with Mesenchymal Stem Cells (MSC) to regenerate the tissue. This method provides a novel strategy to obtain organ scaffolds, but further studies are needed to extrapolate this technique to human transplant.

Keywords
Decellularization, MSC, kidney scaffold, tissue engineering.

1 Introduction

Chronic kidney disease (CKD) is a general term used for heterogeneous disorders affecting kidney structure and function [3]. This disease affects over 25% of individuals older than 65 years [4] and most of them require organ transplantation after a few years of treatment. Due to the increasing number of patients with CKD, the median wait time for a suitable transplant is 3.6 years [5], reason why engineering of kidney tissues *in vitro* from cultured autologous stem cells arise as a promising solution to this medical problem [6].

Bone marrow mesenchymal stem cells (MSC) are a niche of cells with the property of being able to differentiate into almost any end-stage cell type. Easy extraction methods have been developed for this niche and their differentiation properties enable their seeding in specific scaffolds for the study of tissue regeneration [7]. These methods together with kidney decellularization techniques had been already developed in animal models rising the rate of survival in transplanted rats [8].

During the last decades, modern tissue engineering strategies are focusing on production of acellular kidney scaffolds, utilizing cost-effective decellularization strategies [9]. One of these techniques is based on the use of detergents or enzymes which are perfused in an antegrade fashion from the renal artery through the kidney vasculature. The extracellular matrix (ECM) that remains after the decellularization process maintains the delicate glomerular and tubular structures, as well as the vascular tree of the kidney [10] which increases the possibility of using decellularized kidneys from other species as a source of scaffold for transplantation.

A detergent decellularization process was applied to a rat kidney provided *in vivo* in order to obtain the extracellular matrix scaffold and pursue their subsequent recellularization with bone marrow mesenchymal stem cells. This method provided as a final result an organ implant prototype.

This novel technique could be extrapolated to surgery rooms where cadaverous kidneys could be decellularized in laboratories and discarded donated kidney as a scaffold to finally recellularize it with recipient's native cells in order to avoid immune system reactions. Nevertheless, there is still function properties that should be further studied for the transplantation

process [11].

2 Materials and methods

2.1 Perfusion decellularization of kidneys

A rat kidney was isolated for perfusion decellularization. All animal experiments were performed in accordance with the Animal Welfare Act and approved by the institutional animal care and use committee at the University of Carlos III.

A male Sprague-Dawley rat was used. A median laparotomy was performed to expose the kidney capsule, we transected the renal artery, vein, and ureter to retrieve the kidney from the abdomen. We cannulated the portal vein with a 20-24-gauge cannula and immobilized it with a 4-0 silk suture.

Subsequently, the cannula was connected to a peristaltic pump and we started perfusion with 500ml of deionized water. Afterwards, we perfused 4000ml of decellularization solution (Triton X-100 1 percent + NH4OH 0.1 percent) overnight. Following decellularization, we washed the kidney scaffolds with 8000ml of deionized water.

2.2 Rat BM-MS cell isolation

A Sprague-Dawley rat was euthanized for organ harvests. We then excised the left femur, and we transferred it to a sterile Petri dish, and washed it with PBS in order to remove the residual connective tissue. Then we cut the diaphysis from the epiphysis using scissors and moved the epiphysis to a clean Petri dish. Using a syringe with a needle, we passed 5 ml of DMEM/F12 + 10 percent FBS + antibiotic through the epiphysis to flush the bone marrow. We proceeded to recollected and transf the medium to a sterile 15 mL conical tube and adjusted the volume to 10 ml to pass the sample through a 100 µm cell strainer into a 50 ml conical tube.

The resulting cell solution was centrifuged (300g, 5-minutes), and cell pellets were resuspended in 2 ml of DMEM/F12 + 10 percent FBS. The resulting BM-MSC were plated onto 6 well plates (2ml/well) previously coated with 0.2 percent gelatin solution. and kept in an incubator at 37°C in a 5 percent CO2 atmosphere.

2.3 Cell expansion and seeding

Every 2 days and after observing the cell confluence in a inverted light microscope, we removed the spent medium and washed the cell monolayer with 1ml of PBS. Then we added 0.5ml of 0.05washed cell monolayer and incubated it for 2-10 minutes or until cells are detached.

The resuspended cells were placed in a small volume of fresh serum and transferred into a 15 ml conical tube to Centrifuge the sample at 1200 rpm for 5 minutes. The pellet obtained was resuspendeded in fresh media and the cells were counted using a hemocytometer with trypan blue in order to determine cell viability (Formula bellow). The cells were seeded in a 10cm or a 15cm tissue culture dish and incubated at 37°C. $PercentageCellviability = totalviablecells(unstained)/totalcellsx100$

To seed the cells we trypsinized and diluted the BM-MSC in 5 mL of DMEM/F12 + 10 percent FBS + antibiotic and seeded these onto the acellular kidney scaffold via the portal vein cannula at constant flow and cells were allowed to attach.

2.4 Bioreactor design and whole organ culture

We designed and custom built the kidney bioreactor as a closed sterilized system (**Figure 13**). The tubing was connected to the 3-way valves using male and female luer locks plastic fittings. The tubing was connected to all three ports of the bioreactor vessel, and it was primed with culture medium with a 30ml luer lock syringe until all the air was removed from the system. The kidney was attached to the inlet port tubing via cannula through the portal vein and then was suspended in seeding medium within the bioreactor vessel. Perfusion media and cell suspensions were infused at 3 ml/min overnight through the peristaltic pump access ports at 37°C.

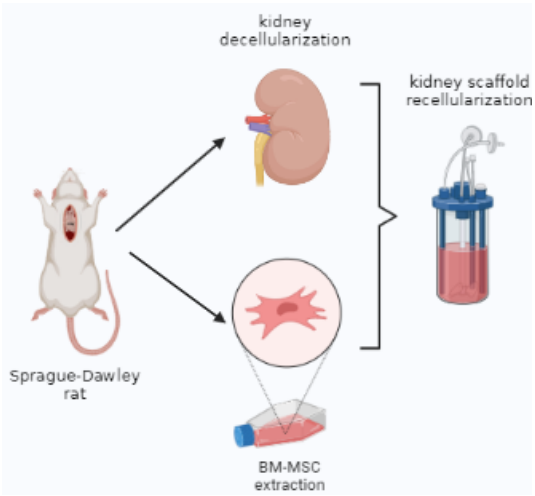

Figure 1. Graphical scheme of the experiment. The picture describes the three major steps: BM-MSC extraction, kidney decellularization and scaffold recellularization in a bioreactor.

3 Results

3.1 Stem Cell Harvesting, Isolation and Expansion

The first step of this technique was to retrieve the MSC of the Sprague-Dawley rat. These cells were obtained from the bone marrow found on the rat's femur. This technique first required an incision that would expose the femur, allowing us to dislocate the femoral head from the acetabulum **(Figure 2)**.

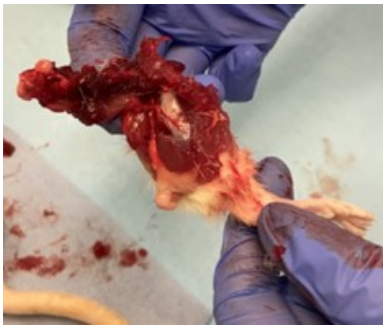

Figure 2. Exposed femur

The collected rat's femur was cleaned before the extraction of the bone marrow.

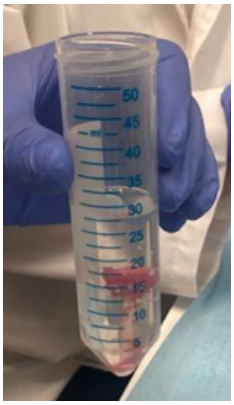

Figure 3. Clean rat femur

At the end of the tube, the femur was entirely preserved: it still presented the diaphysis and the epiphysis.

The diaphysis was cut from the epiphysis, then the epiphysis was placed on a Petri Dish where the bone marrow was flushed using a syringe with 5 ml of DMEM/F12 + 10 % FBS + antibiotic.After this step, the MSC were obtained **(Figure 5)**, which were expected to expand afterwards.

After harvesting the cells from the bone marrow, we isolated the cells in a Petri dish.

At the beginning, here were the cells appearance:

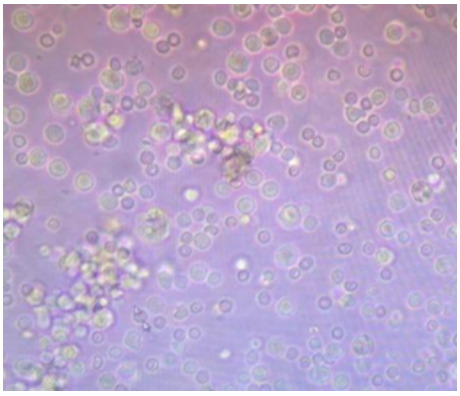

Figure 4. Mesenchyme Stem Cell Day 1

After the BM-MSC on the plate were coated in gelatin and placed in the incubator at 37°C, the cell expansion began. In this step, many cells were observed, but few of them were mesenchyme cells.

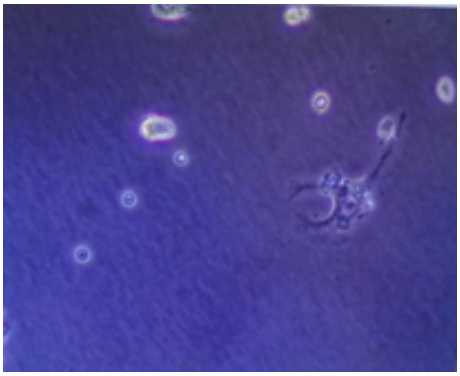

Figure 5. Mesenchyme Stem Cell Day 2

On the right, we observed a mesenchyme cell. On the second day, the cell culture was observed with an inverted microscope in order to evaluate cell expansion. On this day, less than 50 % of the plate was covered by cells.

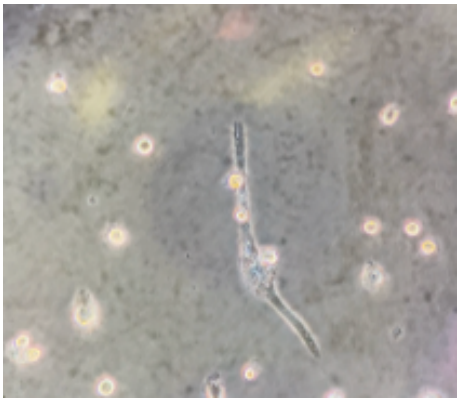

Figure 6. Mesenchyme Stem Cell Day 3

The elongated shape on the right of the picture corresponds to mesenchyme cells.

The MSC fixated on the bottom of the Petri dish, where the gelatin was found; thus the cell monolayer was washed out with PBS and then vacuumed.

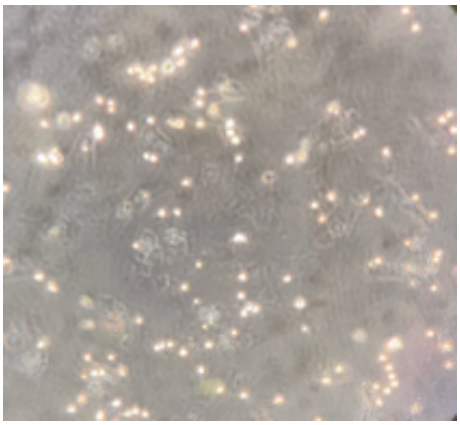

Figure 7. Cell expansion

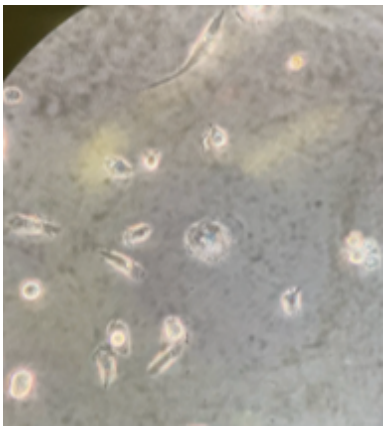

Figure 8. Several MSC

No bacterial or fungal contaminants were observed. After removing the spent medium and washing the cell monolayer with PBS, the serum was removed, and trypsin was added. After incubation, cells were seen detached and floating.

The number of non-viable (entirely stained blue) and viable cells in a $1mm^2$ area was counted by using a hemacytometer. When the cell was entirely stained blue, it was dead (non-viable). The counting process was performed for two areas on the hemacytometer, whoch were labelled as region A and region B. For example, in the first square of the first region, there were 2 viable cells and 1 non-viable cell. In the second one, there were 4 viable cells and 2 non viable cells, and so on.

For region A, the average number of cells was:
avg_x1 = 2.75 cells/ml. **(Equation 1)**
For region B it was:
avg_x2 = 2.25 cells/ml.**(Equation 2)**
Therefore, the average cell count per square was:
avg_x = 2.5 cells/ml. **(Equation 3)**

Total cells

The total number N of cells on the whole hemacytometer is: $N = avg\_x*$ original volume of cell suspension from which the sample was removed. **(Equation 4)**

$N = avg\_x * 3 * 2.10^4$ **(Equation 5)**

$N = 165,000$ **(Equation 6)**

By performing the calculations, it was determined that there were approximately 165,000 cells on the hemacytometer.

Cell viability percentage P

P = total viable cells/total cells * 100 **(Equation 7)**

For area A, there are 9 viable cells out of 13 cells.
P = 9/13 * 100 = 69 %. **(Equation 8)**

For region B, there are 11 viable cells out of 14 cells.
P = 11/13 * 100 P = 85 %. **(Equation 9)**

3.2 Kidney scaffold generation

First, we remove one intact kidney from the rat and confirm that no additional attachments to the liver are present.

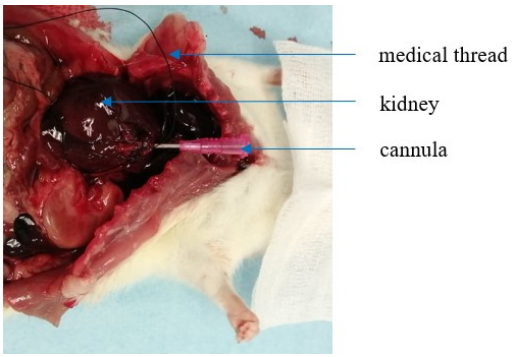

Figure 9. Removal of the kidney

Once the cannula was inserted into the artery, the kidney was placed in water, and it was then perfused with distilled water by connecting the cannula to a peristaltic pump, thus starting the decellularization process.

After perfusion, the kidney was removed from the pump, but the cannula was maintained in the organ to connect it to the bioreactor.

Figure 10. Kidney in a tube

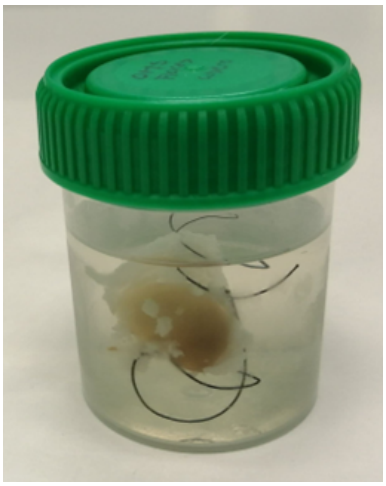

Figure 11. Unseeded bioscaffold

3.3 Kidney bioengineering using a perfusion bioreactor

We assembled a perfusion bioreactor providing physiological preconditioning. The kidney was attached to the inlet port tubing via a cannula in the portal vein and then was suspended in seeding medium within the bioreactor vessel. The bioreactor assembly was transferred to a 37°C incubator and attached to the peristaltic pump.

In parallel, we harvested the BM-MSC from the tissue culture dishes, neutralize with complete media and centrifuge at 1500 rpm for 5 minutes.

The next step was to infuse the scaffold with the stem cells we grew in the culture (Petri dish).

As showed in the picture, we had to inject the cell suspension in the bioreactor vessel through the smart site connector (a). Then perfusate circulated through the tubes, following the blue arrows.

Blue ink was added to visualize the perfusate going through the kidney.

This method permitted us to see cell injection throughout the kidney scaffold.

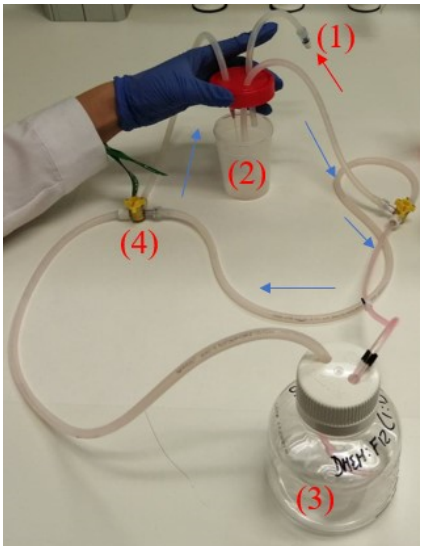

Figure 12. Seeding perfusion bioreactor
(a) cell injection port
(b) bioreactor vessel
(c) reservoir
(d) valve
The blue arrows indicate the direction of circulation.

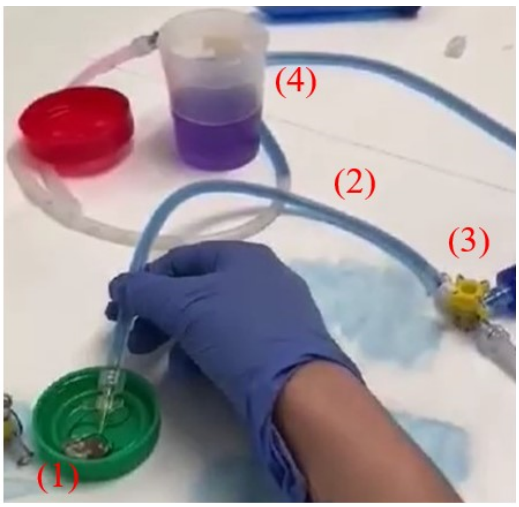

Figure 13. Perfusion of ink

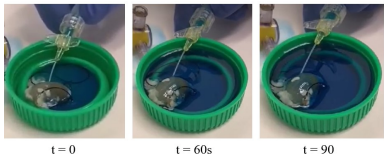

Figure 14. Coloration of the perfused kidney

3.4 Tissue Construct Analysis

Once the recellularized scaffold was obtained, it must be characterized. When doing so, the following parameters should be analyzed:

- ECM composition (before and after)

- DNA content (before and after)
- Macro- and microarchitecture
- Vascular network assessment
- Biocompatibility
- Detergent remnants (biomaterial preparation reagent contaminants)

The removal of cellular components or nuclei and the preservation of tissue architecture could have been verified by a histological analysis. The elastic fiber network of the renal artery should have also been verified.

The Extracellular Matrix (ECM) should have been mainly composed of macromolecules and minerals,including Laminin and Collagen, whose presence could have been highlighted by immunostaining.

The perfused Mesenchyme Stem Cells were supposed to proliferate and differentiate after injection into the kidney scaffold.

Concerning the cells, the following parameters should have been analyzed:

- Flow cytometry
- Functional assays (protein secretion, metabolism, etc)
- Cell differentiation (stem cell to mature cell)
- Gene expression
- Immunofluorescence

The 3 categories of analysis are destructive, semi-destructive, and non destructive. The characterizations of interest are gene expression and protein expression, which are destructive analysis. Moreover, the perfusion bioreactor allowed us to perform a metabolism analysis, which is a non destructive analysis.

4 Discussion

The most commonly harvested cells present in the bone marrow are MSC. To obtain these cells, the femur of the rat was extracted to obtain the highest amount of bone marrow possible. After harvesting, it was seeded on a culture dish with DMEM/F12 + 10% FBS. After this, cell expansion was expected, but in our case, it did not happen since the culture appeared to be contaminated (filled with fungi). To continue with the experiment, we were provided with a sample of cells from another group. Therefore, from this point on the new cells are being discussed.

The culture was observed for several days to confirm cell expansion and viability. To do so, the number of viable and non-viable cells were counted. These cells were differentiated by their color, since non-viable cells were stained with blue pigment whereas viable ones were not. After counting cells, several calculations were made to evaluate global viability.

Decellularization is known as the process of removing cells and cellular components of a tissue from a specific organ selected by the researcher. For this experiment, our targeted organ for decellularization and recellularization was the kidney of a rat. During the process of the kidney removal, it was important to insert the cannula directly into the artery to adequately perfuse it with deionized water **Figure 13**. The expected outcome was for the kidney to be transparent. Our results showed a kidney with a white-ish color **Figure 15**; thus, it was assumed that cellular matters remained in the organ. A possible explanation for this could be that the cannula was not securely placed in the organ, leaving space for error since it was not being perfused with the required parameters.

A bioreactor was assembled to provide preconditioning to the kidney and to be able to inject it with the MSC cultivated in the Petri Dish. The latter one, would be the recellularization process for the organ. The bioreactor was put to work and the cells entered the scaffold; media perfusion was maintained for a few minutes. To have a visual marker during this recellularization process, blue coloring was added into the solution. Kidney recellularization was achieved, as the whole kidney changed from a white-ish color to a blue-ish color.

After analyzing our results, it can be assumed that to further extend our research the function of the organ could have been tested, in this case, filtration. Also, the recellularized kidney scaffold could have been cultured in a biomimetic culture. A new injection through the bioreactor port would provide oxygenation, nutrient supply, and a filtration stimulus for a few days. Afterwards, the output of the kidney would be observed in order to confirm the differentiation of stem cells into nephrons. A histological analysis could evaluate the repopulation of kidney scaffold with epithelial cells.

Acknowledgements

We give out thanks to Pedro, Marta and Angelica for sharing their experience and knowledge with us, making the development of this method possible.

References

[1] Health Resources Service Administration. (2022, February). Organ Donation Statistics, Detailed description of data. HRSA. Retrieved December 9, 2022, from https://www.organdonor.gov/learn/organ-donation-statistics.

[2] Song JJ, Guyette JP, Giplin SE, Gonzalez G, Vacanti JP, Ott HC. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. Nat Med. 2013 May;19(5):646-51. doi: 10.1038/nm.3154. Epub 2013 Apr 14. PMID: 23584091; PMCID: PMC3650107. [3] M. H. Little, S. V. Kumar, and T. Forbes, "Recapitulating kidney development: Progress and challenges," vol. 91, pp. 153–168, Jul. 2019, doi: S1084-9521(17)30426-3 [pii].
[4] B. Wilm, R. Tamburrini, G. Orlando, and P. Murray, "Autologous Cells for Kidney Bioengineering," vol. 3, pp. 207–220, 2016, doi: 10.1007/s40472-016-0107-8.
[5] C. C. Miranda, M. R. Gomes, M. Moço, J. M. S. Cabral, F. C. Ferreira, and P. Sanjuan-Alberte, "A Concise Review on Electrospun Scaffolds for Kidney Tissue Engineering," vol. 9, no. 10, p. 554, Oct. 2022, doi: 10.3390/bioengineering9100554.
[6] K. McCullough et al., "Measuring the population burden of chronic kidney disease: a systematic literature review of the estimated prevalence of impaired kidney function," vol. 27, no. 5, pp. 1812–1821, May 2012, doi: 10.1093/ndt/gfr547.
[7] D. Hernández et al., "Waiting List and Kidney Transplant Vascular Risk: An Ongoing Unmet Concern," vol. 45, no. 1, pp. 1–27, 2020, doi: 10.1159/000504546.
[8] Y. Han, X. Li, Y. Zhang, Y. Han, F. Chang, and J. Ding, "Mesenchymal Stem Cells for Regenerative Medicine," vol. 8, no. 8, p. 886, Aug. 2019, doi: 10.3390/cells8080886.
[9] J. J. Song, J. P. Guyette, S. E. Gilpin, G. Gonzalez, J. P. Vacanti, and H. C. Ott, "Regeneration and experimental orthotopic transplantation of a bioengineered kidney," vol. 19, no. 5, pp. 646–651, May 2013, doi: 10.1038/nm.3154.
[10] P. Mallis, C. Oikonomidis, Z. Dimou, C. Stavropoulos-Giokas, E. Michalopoulos, and M. Katsimpoulas, "Optimizing Decellularization Strategies for the Efficient Production of Whole Rat Kidney Scaffolds," vol. 18, no. 4, pp. 623–640, Aug. 2021, doi: 10.1007/s13770-021-00339-y.
[11] A. S. Levey and J. Coresh, "Chronic kidney disease," vol. 379, no. 9811, pp. 165–180, Jan. 2012, doi: 10.1016/S0140-6736(11)60178-5.