BOHR Journal of Biocomputing and Nano Technology
2023, Vol. 1, No. 2, pp. 30–33
DOI: 10.54646/bjbnt.2023.06
www.bohrpub.com

METHODS

Implanting decellularized plant tissue containing CAR-T cells at debulked tumor sites

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Received: 11 May 2023; **Accepted:** 24 May 2023; **Published:** 31 May 2023

Surgical treatment of cancer includes the removal of the entire tumor. Sometimes, surgery removes some, but not all, of cancer tumors regarding the safety of the organ. This is called debulking. The debulked site contains some cancer cells. CAR-T cell therapy is a cancer immunotherapy that uses T cells of patients to fight against cancer cells. T cells collected from patients' blood are genetically modified using chimeric antigen receptor (CAR) gene. The modified T cells are grown in a culture medium. Decellularization is the process of sterilizing a preexisting natural organ to the extent that only the extracellular matrix scaffold base remains. The CAR-T cells injected decellularized plant tissues are used as an effective treatment against cancer cells present at debulked tumor sites.

Keywords: cancer, tumor, surgery, debulking, CAR-T, T cells, chimeric antigen receptor (CAR), extra cellular matrix scaffold

Introduction

Radiation, chemotherapy, and surgery are the most commonly used cancer treatments. Targeted therapy, immunological therapy, laser, hormonal therapy, and other methods are also available. Many forms of cancer are commonly treated with surgery. Surgical treatment is done by two methods, namely, the entire removal of tumor or partial removal of tumor. The partial removal of tumor will not ensure the complete removal of the cancer cells. Some cancer cells may present at the tumor sites. This is called debulking. The term "chemotherapy" ("chemo") is frequently used to describe treatments for cancer. However, not all cancer medications function in the same way. The medications used in conventional or traditional chemotherapy are cytotoxic, which means they can destroy tumor cells. Knowing how traditional or standard chemotherapy functions and what to anticipate may frequently assist you in preparing for treatment and, if necessary, in making sensible healthcare decisions. Immunotherapy is a type of cancer treatment that helps your immune system fight cancer. Your body's immune system works to keep you healthy and free from infections and diseases. Organs, lymphatic system components, and white blood cells are some of its elements. One example of a biological treatment is immunotherapy. Biotherapy is a type of cancer treatment that uses elements taken from living things to fight the disease. Tumor antigens, which immune system antibody proteins can identify and bind, are often found in cancer cells, which make cancer immunotherapy possible. Frequently, proteins or other large molecules serve as tumor antigens (e.g., carbohydrates). Modified immunotherapy antibodies, in contrast to conventional antibodies, which bind to external pathogens, recognize and mark cancer cells for the immune system to suppress or kill.

Decellularization

Plant decellularization

A nearby store provided the spinach and parsley. Leafy *Artemisia annua* plants that were growing on the ground

were gathered. Agrobacterium rhizogenic- mediated genetic transformation produced peanut hairy roots. Techniques for full organ perfusion and decellularization were tailored for the diverse plant kinds. Cannulas were inserted via the stem segment's basipetal end into the parsley stems and spinach leaves. The plants' cuticles were removed by sequential applications of hexane and 1 phosphate-buffered saline. After being infused, cannulas were infused with a 10% sodium dodecyl sulfate (SDS) in deionized water solution after being treated for 48 h with 0.1% Triton X-100 in a 10% sodium chlorite bleach in deionized water solution for 5 days. For the next 48 h, sterile deionized water was infused. The flow was initiated by gravity and perfused at 152 mmHg of steady head pressure. *Artemisia annua* and peanut hairy roots were decellularized using the same procedure, but this time the roots were immersed in the solutions rather than cannulated and perfused. After being decellularized, it could take up to 2 weeks before tissues were required. They were then kept at 4°C in sterile, deionized water.

Histological analysis

The major vascular path of the leaf was sliced down the center of each square. Ideally, the leaf samples were cut into 1 cm squares. Trim the roots and stems into pieces that were about 1 cm long. After being treated for the night, tissue samples were paraffin embedded in the automatic tissue processor ATP-1. Paraffin blocks were divided at a depth of 14 m. Materials were handled using the Fast Green method and Sass's Safranin to stain plants, as was already indicated. Slices were stained for 1 h in an aqueous solution containing 1% (w/v) Safranin-O, followed by a 5-min thorough rinsing in deionized water to completely eliminate all remnants of the dye. Sections were dehydrated for 10 s in 70, 95, and finally 0.1% w/v Fast Green FCF in 95% ethanol. Sections were cleaned twice in xylene for 2 min each after being rinsed twice in 100% ethanol for the same amount of time. Samples were lignin-stained for 10 days as previously mentioned, using a saturated solution of phloroglucinol in 20% HCl. The samples were viewed using a DMLB2 upright microscope.

Preparation and characterization of decellularized plant scaffolds

Because of the fundamental architecture of higher plants, nutrients can be transported to distant cells by way of xylem and phloem, for example, from roots to leaves, then from leaves to additional leaves or additional roots. *Spinacia oleracea* (spinach) leaves were modified for perfusion decellularization in order to investigate the possibility of a plant-based tissue engineering scaffold. Due to their widespread availability, the pattern and density of their vascular network, and the size of their petioles, the model species used was spinach leaves. After being cannulated, spinach leaf petioles were perfused for 5 days. After 2 days of treatment with a clearing agent (10% SDS in deionized water), the decellularization solution should be applied. One day after the decellularization process began, the leaves began to lose their green hue due to the loss of chlorophyll, which represents the loss of chloroplasts from the leaf tissue. By day 5, the leaves were translucent and had a green hue. A colorless, translucent leaf was produced after the tissue was sterilized and any remaining chlorophyll was removed by adding sodium chlorite. Native leaves were found to have cells with nuclei and chloroplasts through histological investigation, whereas their decellularized counterparts did not. Lignin, a substantial. According to further histological labeling, the leaf vasculature's biopolymeric component was discovered both before and after decellularization. Decellularization of spinach leaves did not change the topography of the leaf's surface, as shown by scanning electron microscopy scans that showed the same vascular network pattern and density as natural leaves. The leaves could not be fully decellularized by decellularization or clearing solutions, indicating the potential need for anionic and non-ionic detergents to entirely remove all plant cellular waste.

CAR-T cell therapy

The goal of CAR-T immunotherapy is to modify T cells so that they can recognize cancer cells, making it easier to find and eradicate them. Researchers genetically modify human T cells by adding a chimeric antigen receptor (CAR) that selectively recognizes cancer cells. Patients are subsequently given an injection of the generated CAR-T cells to combat their tumors.

Immune receptors and foreign antigens

By scanning the surface of those cells for proteins known as antigens, the immune system may identify foreign substances in the body. T cells, which are immune cells, have proteins on their own called receptors that connect to foreign antigens and aid in causing the other immune sys- tem components to start destroying the foreign substance. Antigens and immunological receptors work together like a lock and key. Each foreign antigen has a particular immune receptor that can attach to it, similar to how a lock can only be opened with the proper key. Cancer cells also have antigens, but if your immune cells lack the proper receptors, they would not be able to bind to the antigens and aid in the removal of the cancer cells.

Chimeric antigen receptors (CARs)

CAR-T cell treatment uses T cells that are drawn from the patient's blood, and a gene for the CAR, or chimeric antigen receptor, a receptor that helps the T cells in adhering to a particular cancer cell antigen, is added to the T cells in a laboratory setting. The patient then receives their CAR-T cells in return. Each CAR is created for a particular cancer antigen since many malignancies have various antigens. For instance, cancer cells in certain forms of leukemia or lymphoma express the antigen CD19. Because CAR-T cell treatments are unsuccessful against cancer since they are created to bind to the CD19 antigen in malignant tumors, the CAR-T cell treatment might go on for several weeks.

Collecting the T cells

Leukapheresis is a procedure used to first remove T cells and other white blood cells from the patient. During this procedure, patients often lie in bed or recline in a chair. Since one line is used to remove blood and the other to remove white blood cells before reintroducing blood to the body, two IV lines are necessary. Sometimes, a central venous catheter—an IV line with both IV lines built in— is used. Throughout the process, the patient must be still for 2–3 h, either sitting or lying down. Leukapheresis can occasionally result in a decline in blood calcium levels, which can result in tingling and numbness as well as muscle spasms. To treat this, the calcium can be replaced by giving it orally or intravenously.

Making the CAR-T cells

The T cells are isolated after the white cells have been harvested and transferred to the lab, where the gene for the particular CAR was inserted. CAR-T cells are the result. Then, in a lab, these cells are multiplied and cultivated. Making the enormous number of CAR-T cells required for this therapy can take weeks.

Receiving the CAR-T cell infusion

Chimeric antigen receptor T cells will be returned to the patient once there are enough of them. The patient might get chemotherapy a few days before to the CAR T-cell infusion to assist reduce the amount of other immune cells. The likelihood that cancer- fighting CAR T cells will activate is increased by this. This treatment is often not particularly potent since CAR T cells work best when there are still cancer cells to attack. The CAR T cells begin to multiply and potentially aid in the destruction of additional cancer cells once they begin binding with cancer cells.

Infusing CAR-T cells in decellularized plant tissues

Infusing the CAR-T cells into decellularized plant tissue and implanting it in the debulked tumor site bridges the gap between surgical and immunotherapy cancer treatment. As the plant's tissue vascular system almost resembles the human vascular system, providing nutrients to the CAR-T cells becomes easy.

Merits of using decellularized plant tissues

A method for the *in situ* engineering, replication, and release of genetically altered T cells is known as "Multi- functional Alginate Scaffolds for T Cell Engineering and Release" (MASTER). A breakthrough has occurred in the field of CAR-T cell therapy. T cells from the patient are extracted and combined with an engineered virus that contains a gene that targets malignancy (as with CAR-T). A MASTER (scaffold) is then used to absorb them when the mixture is introduced. Interleukins and antibodies found in the MASTER cause cell division and T-cell activation, respectively. The patient is then given the MASTER. The viruses interact with the activated T cells to create CAR- T cells. These CAR-T cells are encouraged to multiply by the interleukins, and once they have left the MASTER, they target the cancer. Instead of taking weeks, the method only needs hours. Additionally, because the cells are younger, they remain in the body longer, have greater resistance to cancer, and exhibit fewer signs of weariness. Mouse models were used to demonstrate these characteristics. The lymphoma treatment was more successful and long lasting. Compared to master decellularized plant tissues, it can be used for better structural stability as it provides the extracellular matrix as a scaffold.

(1) It has been demonstrated that cellulose is biocompatible and encourages wound healing. Additionally, it was discovered that biocompatible cellulosic tis- sue engineering scaffolds made from decellularized apple slices could support mammalian cell adhesion and proliferation when implanted subcutaneously *in vivo*. Pectin and hemicellulose are also being researched as potential biomaterials for bone tissue engineering and wound healing, respectively. Due to their intrinsic similarities and apparent biocompatibility of their ECM, we investigated whether plants and their native vasculatures could serve as perfusable scaffolds for developing human tissue. Decellularization of a range of plant species and tissues produced acellular, vascularized scaffolds for tissue engineering. Numerous plant species' profusion and quick development also make them an excellent

source for a less expensive, more accessible, and long-lasting scaffold material.

(2) White blood cells (leukocytes), as well as some other body cells, express and secrete a group of proteins and signal molecules called interleukins. More than 50 interleukins and associated proteins are encoded by the human genome. Interleukins are primarily.

(3) Responsible for the immune system function, and some of them have been reported to be deficient in cases of autoimmune disorders or immunological deficiencies. Monocytes, macrophages, endothelial cells, and CD4 helper T lymphocytes are responsible for the majority of interleukins' synthesis. They support the growth and differentiation of hematopoietic cells, including T and B lymphocytes. Interleukins' ability to promote the growth of CAR-T cells will enhance the treatment even more. These T cells when interacted with the tumor cells produce CARs on their cell membrane and attack the cancer.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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