

Arthritis affects 4 million Canadians and osteoarthritis is the most common type of arthritis. It is the leading cause of work disability in Canada with a cost of \$4.4 billion per year. My research is focused on the biologic restoration of joint surfaces to prevent the development of osteoarthritis. There are a number of reasons why a joint surface can require restoration including trauma, tumours, and avascular necrosis. Our moveable joints are lined with a complex tissue called articular cartilage that provides almost frictionless range of motion and decades of function. When a portion of the joint surface is damaged, especially if greater than 1cm in diameter, the whole joint is likely to deteriorate over time resulting osteoarthritis with pain, stiffness, and loss of function. Once osteoarthritis begins, there is no treatment that can reverse the process. Eventually, osteoarthritis can be treated with a synthetic total joint replacement but there remain significant limitations to that surgery depending on the patient's age and activity level. Therefore, it is paramount to prevent the development of osteoarthritis at an early stage. Currently, there are some methods that can regenerate a type of cartilage that provides some function but does not regenerate the complex properties inherent in articular cartilage. In addition, these techniques are generally limited to smaller areas. One alternative is to take articular cartilage from another joint. Unfortunately, we do not have spare cartilage to take in significant quantities so it must be taken from decreased donors, especially for larger lesions. This is currently in practice but there are still limitations because the storage time after harvest is limited to 28 days during which the health of the tissue is compromised. Even by 7 days, the tissue starts to deteriorate. This short time frame makes it very difficult to assemble the surgical team, match for size and location and test for infectious diseases resulting in suboptimal transplantation conditions and the wastage of tissue that is harvested but not transplanted within 28 days.

I am involved in research that has investigated ways to allow storage of articular cartilage for prolonged periods of time without tissue deterioration. This research is multi-disciplinary with my two main collaborators being Dr. Locksley E McGann (physicist) and Dr. Janet AW Elliott (thermodynamicist). To accomplish this, we have focused on a method of cold preservation called vitrification. Vitrification is the formation of a solid from a water solution *without* the formation of ice crystals. Storage at above 0°C results in continued cellular metabolism and eventually cellular decline. Storage below 0°C without special treatment results in ice formation that kills the cells and damages the matrix. Vitrification uses cryoprotective agents such as DMSO and glycerol in very high concentrations to block the water molecules from forming ice crystals. This special solution is rapidly cooled to very low temperatures (usually below -145°C) to stop molecular movement. This results essentially in suspended animation. The cells in these conditions are alive but not aging as biochemical and biological functions stop. These cells can be store in this condition indefinitely. Once the tissue is warmed up and the cryoprotective agents are removed, the cells resume function as before they were vitrified. Thus, successful vitrification can store articular cartilage while keeping the cells viable and preventing ice from damaging the cells and the cartilage matrix.

There were three main obstacles to achieving successful vitrification of articular cartilage: 1) cryoprotective agent toxicity, 2) cryoprotective agent permeation into the complex matrix and 3) interactions between the cryoprotective agents used. Over the past 10 years, we designed a series of studies to overcome these obstacles. The results from these experiments resulted in 18 manuscripts

Vitrification of intact human articular cartilage Dr. Nadr M. Jomha

directly related to this research with 35 presentations at local, national and international meetings. We took the information generated in this foundational research and applied it to intact human articular cartilage on a bone base. By using low concentrations of multiple cryoprotective agents (low concentrations to limit toxicity but multiple agents to achieve a concentration sufficient to vitrify) and applying these cryoprotective agents sequentially at lowering temperatures (to decrease toxicity) for specified periods of time (as determine to achieve the desired concentration within the tissue), we were able to repeatedly vitrify intact human articular cartilage with good to excellent cell viability. Confirmation of success was determine by using membrane integrity (approximately 75% of the cells remained intact), metabolic function and by removing the cells after warming and culturing them in a specific manner to ensure that the cells can still produce articular cartilage specific macromolecules such as collagen II and sulfated proteoglycans. We are now in the process of optimizing the protocol in an attempt to maximize cell recovery, decrease the processing time and develop methods of packaging so that this technique can be brought into clinical practice. It is our goal to establish vitrification as a viable method for banking articular cartilage on a bone base so that it can be used to resurface/restore damaged joint surfaces.