Cryobiology 66 (2013) 201–209

Contents lists available at SciVerse ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Review

# Cryopreservation of articular cartilage ☆

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ARTICLE INFO

*Article history:*
Received 3 July 2012
Accepted 1 March 2013
Available online 13 March 2013

*Keywords:*
Articular cartilage
Chondrocyte
Cryopreservation
Vitrification
Liquidus-tracking

ABSTRACT

Cryopreservation has numerous practical applications in medicine, biotechnology, agriculture, forestry, aquaculture and biodiversity conservation, with huge potentials for biological cell and tissue banking. A specific tissue of interest for cryopreservation is the articular cartilage of the human knee joint for two major reasons: (1) clinically, there exists an untapped potential for cryopreserved cartilage to be used in surgical repair/reconstruction/replacement of injured joints because of the limited availability of fresh donor tissue and, (2) scientifically, successful cryopreservation of cartilage, an avascular tissue with only one cell type, is considered a stepping stone for transition from biobanking cell suspensions and small tissue slices to larger and more complicated tissues. For more than 50 years, a great deal of effort has been directed toward understanding and overcoming the challenges of cartilage preservation. In this article, we focus mainly on studies that led to the finding that vitrification is an appropriate approach toward successful preservation of cartilage. This is followed by a review of the studies on the main challenges of vitrification, i.e. toxicity and diffusion, and the novel approaches to overcome these challenges such as liquidus tracking, diffusion modeling, and cryoprotective agent cocktails, which have resulted in the recent advancements in the field.



## Introduction

Articular cartilage is the white dense material covering the ends of the bones in the articulating joints, such as the knee. Compared to most other tissue types in the human body, articular cartilage is a simple tissue containing only one cell type, called chondrocytes, with no vascular, lymphatic or nervous system. Articular cartilage consists of a collagen network, predominantly of collagen type II, developed specifically to respond to the mechanical forces on the joint. Packed within this collagen network are proteoglycans that provide the hydraulic-like resistance to mechanical forces. These proteoglycans are hydrophilic resulting in a large proportion of the weight and volume of articular cartilage being water (varying from 65% to 80% depending on the type and depth of the cartilage). Chondrocytes are the lone cell type present in cartilage, and are scattered throughout the matrix with a denser, horizontally aligned distribution close to the contact surface (tangential zone). Further from the surface, the density of chondrocytes decreases and they become randomly distributed. Finally, closer to the tide-mark (bone-cartilage boundary) the cells are more vertically aligned. Chondrocytes produce and maintain the extracellular matrix precursor material including the collagen and the proteoglycans that also change in distribution and orientation from superficial to deep within the cartilage matrix. The absence of a vascular supply in articular cartilage means that the cells receive nutrients and discharge waste material by diffusion through the extracellular matrix, from and to the synovial fluid, respectively. As a result, articular cartilage has a very limited ability to self-heal and joint injuries with articular cartilage damage can lead to cartilage degeneration and subsequent osteoarthritis with significant personal and socioeconomic costs [93].

Cartilage replacement or repair can be indicated for a number of medical conditions including: avascular necrosis/osteochondritis dissecans, traumatic injuries, epiphyseal tumors and arthritis. These conditions do not have good non-surgical or surgical options to restore joint function and, if left untreated, they lead to joint instability, deterioration and subsequent osteoarthritis. Arthritis is the most common cause of disability in North America and osteoarthritis is the most common form of arthritis. More than 20 million people in the US alone deal with severe limitations in function on a daily basis due to arthritis, which results in more than 1 million hospitalization cases, and costs a total of $100 billion US every year [1].

Surgical treatment options depend on the type and size of the cartilage lesion. Small lesions less than 1 cm in diameter typically can be compensated by the surrounding cartilage but not always.

☆ *Statement of funding:* The preparation of this review article was funded by the Canadian Institutes of Health Research (CIHR MOP 86492).

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0011-2240/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cryobiol.2013.03.001

Persistently symptomatic smaller lesions and larger lesions often require surgical intervention. The most common cell-based interventions are microfracture [97,98], autologous chondrocyte implantation (ACI) [15,16], and matrix associated ACI (MACI) [11,66]. There are many reports of these surgical techniques providing some positive results but they are unable to reproduce the complex structure of the articular cartilage matrix resulting in biomechanically inferior fibrocartilage or "hyaline-like" cartilage. It is possible that these inferior cartilage repair tissues will not function well in the long-term. Another surgical treatment option is mosaicplasty (osteochondral autografts) which consists of taking cartilage from one area of the joint and moving it to the defect. This will restore the normal cartilage matrix structure in the injured area at the cost of removing it from a previously healthy area of the joint. All of these techniques can provide some relief in small to medium size defects but they are not able to treat large defects, including whole joints, effectively. The only biologic technique that can restore partial [102,103], or whole joints [9,19,36] is osteochondral allografting [38] which entails transplanting bone and cartilage from a donor patient into a recipient patient. In older patients, synthetic total joint replacement is a viable option for more sedentary, low activity patients. Unfortunately, this is not as good an option in younger or active patients because the synthetic joint replacements tend to fail after 7–15 years. Osteochondral transplantation is a possible option for these younger, more active patients but access to fresh tissue is extremely difficult.

Fresh osteochondral transplants provide the best case scenario for cell viability and matrix integrity but fresh transplant is fraught with technical difficulties. This tissue should be harvested within 24 h of death of the donor and is typically transplanted within 48–72 h after harvest [37]. This time frame is too short to perform the extensive testing required to rule out the possibility of transmission of infectious diseases. Considering that joint injury and osteoarthritis are not life threatening, the risk may not be warranted. Another significant technical difficulty is that matching for size and contour, which are important factors for long term successful outcomes, is extremely difficult on such short notice [19]. Making arrangements for complicated joint replacement surgery on short notice can result in logistic problems in arranging the operating room, appropriate surgical staff, surgeon and even the patient. Currently, blood/HLA typing is not performed as articular cartilage is considered immune privileged. That said, the cartilage is transplanted on bone and there can be minor immune reaction to the transplanted bone. This is typically self-limited as the transplant bone is replaced with host bone if only a small amount is transplanted. In the future, blood/HLA typing may be employed to limit the immune reaction which adds another layer of complexity to performing this surgery on short notice. To address these issues, hypothermic storage at 4 °C for a limited time (28–42 days) is used to increase the supply [41,110]. Unfortunately, tissue deterioration begins after only 7–14 days [65]. The lack of normal mechanical stimulation impairs the efficiency of nutrient and waste transport, and decreases cytokine secretion (IL-1 and TNF-α) as reviewed by Kim, Teng and Dang [58]. The ability to store articular cartilage indefinitely would allow for precise size/contour matching, pre-surgical planning, testing for infectious diseases, possible blood typing and appropriate surgical timing for the patient, operating staff and surgeon.

Successful cryopreservation of articular cartilage, by either classical methods or vitrification, can extend the availability of the tissue and allow long-term banking of articular cartilage. Successful cryopreservation and banking of articular cartilage will enable easier and more efficient utilization of straightforward protocols for transplantation. From a cryopreservation perspective, articular cartilage with its extracellular matrix containing no lymphatic, nervous or vascular systems and only one cell type is considered to be a stepping stone for the transition from simple cell to complex tissue cryopreservation with high cell viability and function. Information gained from cryopreservation of articular cartilage can provide valuable insight into more complex tissue and organ cryopreservation.

In the following, a review of articular cartilage cryopreservation methods for transplantation is presented. First, the milestones of cartilage cryopreservation research are reviewed in chronological order, and the basics of associated injuries in classical cryopreservation methods for cartilage are discussed. Then, the prospect of vitrification in lieu of classical cryopreservation, and the current status of cartilage cryopreservation are reviewed. At the end, a summary of challenges are presented and viable approaches are discussed.

## Cryopreservation-associated injuries in cartilage

Successful cryopreservation of articular cartilage is difficult to achieve due to general cryopreservation challenges and some cartilage-specific challenges. Tissues are more challenging to cryopreserve than cellular systems in suspension for many reasons. In tissues, both the cellular activity and the matrix structure must be preserved and this is complicated by the intimate relationship of the cells with the extracellular matrix. Tissues generally contain multiple cell types each with different cryopreservation parameters. Furthermore, different tissues have different requirements for transplantation. In some tissues, such as skin or bone grafts, transplantation of the extracellular matrix is preferred without the native cells to decrease the risk of immunorejection in the recipient [12,42]. Alternatively, some tissues such as articular cartilage require the cellular system for proper long-term functioning of the extracellular matrix; therefore, the cryopreservation strategy must be able to minimize the damage to both the extracellular matrix and the cells.

### *Cryopreservation of isolated chondrocytes*

The earliest investigation into the preservation of chondrocytes was done by Curran and Gibson (1956) [22] who investigated the radioactive sulfate uptake of chondroitin sulfate in human chondrocytes as a measure of chondrocyte viability in 0.5 mm thick cartilage slices obtained from rib, ear or nose. They demonstrated that the cartilage can stay viable for up to 40 days in Tyrode solution at 4 °C. However, cartilage slices, untreated or pretreated (with 10% to 30% w/w glycerol solutions), cooled down to −25 °C showed no recovery of the chondrocytes. Heyner (1960) [40] trypsinized the cartilage for 25 min before slow and rapid freezing in 15% glycerol solutions. It appeared that the chondrocytes in trypsinized cartilage could survive slow freezing to −79 °C and grow in culture while the chondrocytes in untrypsinized cartilage could not tolerate freezing temperatures lower than −20 °C. It was concluded that the failure of the chondrocytes to survive freeze–thaw protocols was related to the cartilage matrix and cell-matrix interactions. Subsequent research was performed on isolated chondrocytes to determine their ability to survive freeze–thaw protocols before spending more effort on the chondrocytes *in situ*.

Unsuccessful freezing of cartilage slices by Curran and Gibson [22] (1956), followed by encouraging results from freezing of partially trypsinized cartilage by Heyner [40] (1960), led to cryopreservation of isolated chondrocytes from rabbits, dogs, monkeys and human, by Smith (1965) [94], to avoid complications of dealing with a collagen matrix. Smith replaced glycerol with $Me_2SO$ and cooled the chondrocytes in 10% w/w $Me_2SO$ to −20 °C at −1 °C/min followed by cooling at −4 °C/min to −79 °C, and found that