SEMARAK ILMU PUBLISHING

Journal of Advanced Research in Applied Mechanics

Journal homepage:
https://semarakilmu.com.my/journals/index.php/appl_mech/index
ISSN: 2289-7895

Effect of Storage Temperature of Articular Cartilage on Magnetic Resonance Imaging Greyscale and Biomechanical Properties

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ARTICLE INFO

Article history:
Received 5 October 2024
Received in revised form 6 November 2024
Accepted 12 November 2024
Available online 30 November 2024

Keywords:
Elastic modulus; permeability; low-field MRI; greyscale; articular cartilage

ABSTRACT

Storage and preservation of articular cartilage are important in experimental studies. Various storage temperatures were used which led to conflicting results on the biomechanical properties of the cartilage. Therefore, this study aimed to examine the effect of storage temperature on the magnetic resonance imaging (MRI) image and biomechanical properties of articular cartilage. The cartilage specimens were extracted from hip bovine joints which were then tested in fresh condition and in a single freeze-thaw cycle at -20°C to observe the effect of the temperature. Low-field MRI was utilized to obtain the images which were processed to determine the greyscale intensity of the cartilage. Subsequently, indentation tests were conducted on the cartilage and the data was integrated with finite element analysis to characterize the biomechanical properties of permeability and elastic modulus. Based on the results, the elastic modulus of the frozen cartilage decreased to 29%, while the permeability increased to 77% compared to the fresh cartilage. Furthermore, a strong positive correlation (r=0.85) was found between MRI image greyscale and permeability, while a negative moderate correlation (r=-0.51) was obtained between MRI image greyscale and elastic modulus. These findings highlighted the significant effect of storage temperature on the MRI image greyscale and biomechanical properties of cartilage.

1. Introduction

Articular cartilage is a resilient and smooth tissue that covers the ends of bones in a synovial joint. It has a distinctive structural arrangement that mostly consists of fluid with chondrocyte cells and an

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https://doi.org/10.37934/aram.128.1.4049

extracellular matrix comprised of collagens and proteoglycans [1,2]. The interstitial fluid is composed of water and dissolved electrolytes which play an important role in load-bearing between articulated bones in the joint. Moreover, it also serves as a medium for lubrication and nutrition in the synovial joint [3-5].

The distribution of water content, proteoglycan, collagen concentrations, and chondrocytes is systematized based on the depth of cartilage tissue to regulate the macromolecular environment within the tissue [6,7]. Therefore, it is crucial to store the cartilage in appropriate condition so that the tissue maintains the macromolecular which has significant effects on the physiological properties. Appropriate storage protocols were applied to preserve the cartilage tissue before or throughout an experiment [8,9]. However, various storage protocols were applied in previous studies, knowing that the storage temperature could affect the properties of the cartilage.

The storage temperature varied from 4 ℃ to -80 ℃ at different timeframes and was applied in previous studies to investigate the biomechanical properties of the cartilage [10-12]. It was observed that during the freeze-thaw cycle, the acid-soluble collagens released into the matrix intrinsically caused micro-damage to the triple helix structure of collagen fibers that is sufficient to alter the biomechanical properties of articular cartilage [13,14]. This circumstance leads to alterations in the pH values of the extracellular matrix, thereby affecting the optimal performance of the chondrocytes to maintain tissue metabolism and degrading the viability of the cells over time [14,15].

Low-field magnetic resonance imaging (MRI) has emerged as a promising imaging modality for examining cartilage due to its cost-effectiveness and accessibility compared to high-field MRI systems. The greyscale of the low-field MRI plays a crucial role in assessing the composition and structural integrity of the tissue [16]. Changes in greyscale intensity can indicate alterations in cartilage composition, such as variations in water content or disruptions in collagen structure, which are indicative of degenerative processes or injury [17]. Hence, understanding the relationship between greyscale values in low-field MRI images and the underlying cartilage properties is essential for accurately interpreting imaging findings and diagnosing cartilage conditions [18-20].

Despite previous studies exploring the effect of storage temperature on articular cartilage properties during the freeze-thaw cycle, there is a lack of consensus on the optimal storage conditions to preserve the tissue's macromolecular environment. This is important for optimizing storage protocols and preserving cartilage integrity for accurate imaging assessments and its physiological properties for experimental purposes. Therefore, the objective of this study is to investigate the effects of storage temperature on both the MRI image greyscale and biomechanical properties of cartilage tissue, aiming to provide valuable insights for optimizing storage protocols and preserving cartilage integrity.

2. Methodology

2.1 Specimen Preparation

The bovine hip joints of a four-year-old local breed were obtained from a local abattoir within 24 hours of the livestock execution. Excess flesh and ligaments were discarded using a scalpel, scissors, and an electric handsaw. The cartilage specimens were prepared in two different conditions which were fresh and frozen hip joints. The fresh hip joints (n=6) were processed immediately upon receiving from the local abattoir, while the frozen hip joints (n=6) were frozen at -20°C for seven days before undergoing a single freeze-thaw cycle [9,21].

In this study, only femoral head cartilage from the hip joint was used for the investigation. As depicted in Figure 1, the femoral head was divided into four sections namely, lateral left (LL), lateral right (LR), medial left (ML), and medial right (MR) to prepare the cartilage specimens for fresh joint

(n=24) and frozen joint (n=24). The anterior cruciate ligament (ACL) was set as the reference point since it was visible in the MRI images.

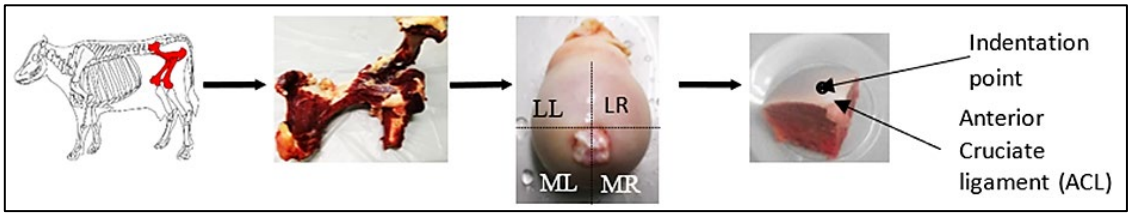
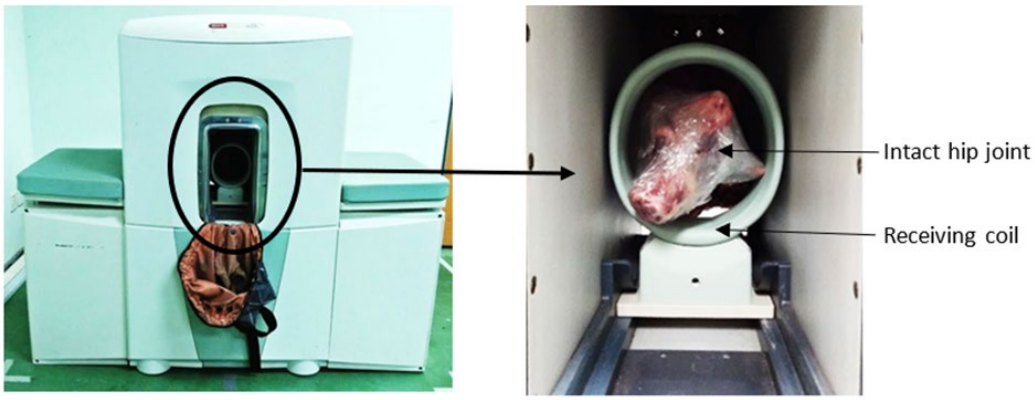


Fig. 1. Preparation of cartilage specimen

2.2 Low-Field Magnetic Resonance Imaging

The intact hip joints were scanned using a low-field 0.2T Esoate C-Scan MRI system (Genova, Italy). The low-field MRI was previously utilized to provide cartilage images in various studies [22-24]. The joint was positioned in the middle of the receiving coil to visualize the acetabulum and femoral head as shown in Figure 2. Gradient echo (GE) imaging sequence was applied to scan the joint where the parameters are shown in Table 1 [25,26].



(a) (b)

Fig. 2. (a) C-scan system on MRI (b) Intact hip joint set up at receiving coil

Table 1
GE Imaging parameters of low-field MRI [25]

Parameter	GE
Repetition time, TR (ms)	2660
Echo time, TR (ms)	18
Thickness (mm)	2
Matrix	256
Field of view (mm)	180

2.3 Assessment of MRI Image Greyscale

The encoded Digital Imaging and Communications in Medicine (DICOM) format MRI images were processed using MATLAB (MathWorks Inc., MA, USA) software. The cartilage images were characterized based on the greyscale intensity that represents white, grey, and black shades in each pixel. The resolution of the MRI image was 12 bits per pixel, resulting in the greyscale values from 0 to 4,096. A rectangular region of interest (ROI) was selected at the indentation test area where each of the ROI consists of 22 pixels across the cartilage thickness to determine the average greyscale.

Figure 3 shows the MRI coronal and transverse views of the ROI.

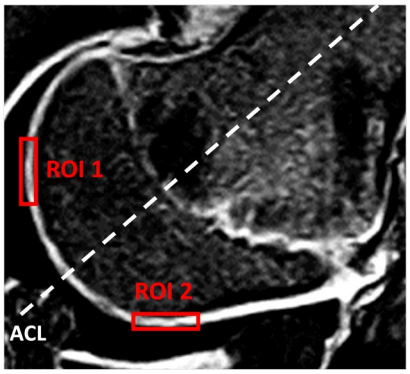


Fig. 3. Low-field MRI image of ROI locations on the femoral head

2.4 Characterization of Biomechanical Properties

The permeability and elastic modulus of the cartilage were characterized by integrating the data from the creep indentation test and finite element analysis. The indentation test was conducted using a 4 mm diameter spherical indenter to indent at the ROI of each cartilage specimen. The test was subjected to 0.38 N compression load which resulted in between 6% to 20% deformation of the cartilage thickness [27]. The displacement was continuously recorded in LabVIEW software at every 0.01 seconds for 2000 seconds as it reached equilibrium. Figure 4 shows the setup of the creep indentation test.

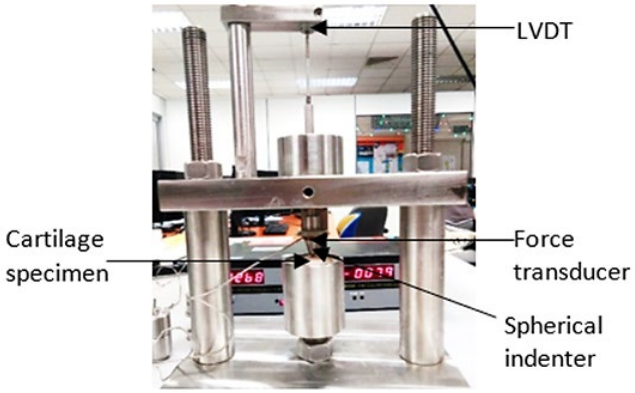


Fig. 4. Creep indentation test setup

Subsequently, the thickness of the cartilage was measured using the indentation apparatus with needle indenter. A load compression of 3.18 N was applied to penetrate the cartilage tissue until it reached the subchondral bone [11,28]. The thickness was calculated based on the distance from the indenter first in contact with the cartilage until the subchondral bone.

In finite element (FE) modeling, an axisymmetric biphasic poroelastic FE model was developed to represent the solid and fluid phases of the cartilage using Abaqus FE software. The cartilage was modeled based on the measured thickness using the pore pressure and displacement four-node bilinear (CAX4P) element, while the subchondral bone was represented by four-node bilinear elements (CAX4) with a Poisson's ratio of 0.2, and elastic modulus of 2000 MPa as described previously [28]. The 2 mm radius spherical indenter was modeled as an analytical rigid surface as shown in Figure 5.

The interface and boundary conditions were applied in the FE model to simulate the experimental creep indentation test [29,30]. The nodes on the axis were constrained in the horizontal direction, whilst the bottom nodes of the bone were constrained in both horizontal and vertical directions. Meanwhile, the spherical indenter was only allowed to move in the vertical direction. In terms of fluid flow, previous contact-dependent flow was applied at the top of the cartilage surface [20]. In addition, fluid flow was prevented at the bottom and the vertical symmetry axis of the cartilage surface. For the outer edge of the cartilage, the nodes were maintained at zero pore pressure to allow unrestricted fluid flow.

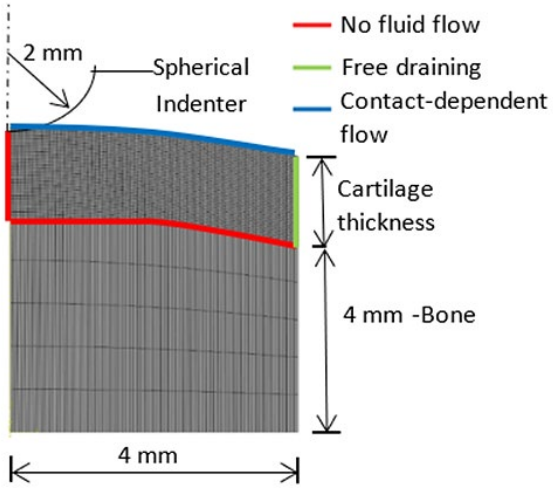


Fig. 5. Axisymmetric FE model of cartilage Specimen

The deformation-time curve generated by the FE model was adjusted iteratively to match the experimental data. The curve-fitting was generated using the non-linear least-square technique in MATLAB software [29]. Permeability and elastic modulus of the cartilage were obtained when the curves fitted satisfactorily.

2.5 Correlation of Biomechanical Properties and MRI Greyscale

The Linear Pearson correlation was used to establish the relation between the permeability and elastic modulus with MRI image greyscale of the cartilage for both fresh and frozen conditions. The correlation coefficient, r, was used to indicate the strength and direction of the correlation relationship.

3. Results

3.1 Effect of Storage Temperature on MRI Greyscale

The effect of cartilage storage temperature on the MRI image greyscale was examined for the fresh and frozen at -20 °C. Distinctive pixel layers were observed in the MRI image of the cartilage which could be classified into superficial and deep zones. The greyscale value at the superficial zone of fresh cartilage was found to be 1340.30 ± 373.41, whilst the frozen cartilage was decreased to 10% at 1210.45 ± 392.97. At the deep zone, the greyscale of the fresh cartilage was 1220.43 ± 422.90 while the greyscale of the frozen cartilage was reduced to 7% at 1129.56 ± 431.63. The comparison between the greyscale of the fresh and frozen cartilage specimens based on superficial and deep zones is shown in Figure 6.

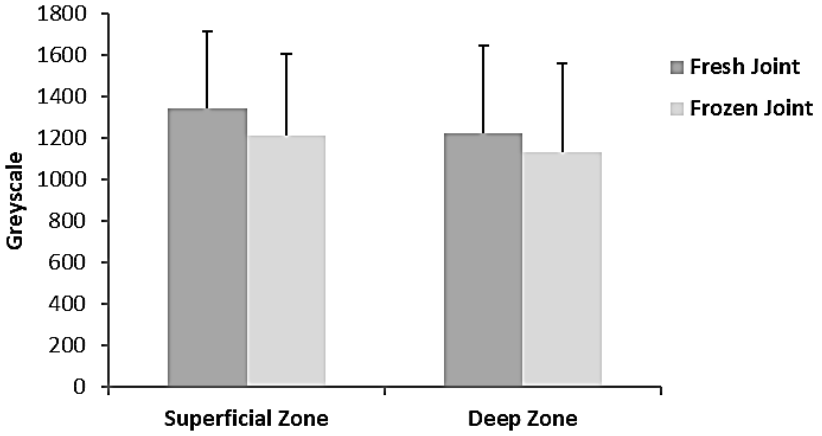


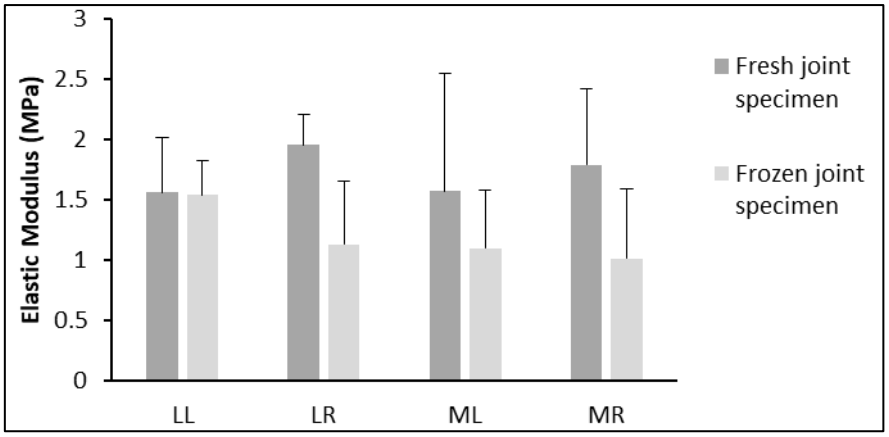
Fig. 6. Low field greyscale on MRI of cartilage at superficial and deep zones for fresh and frozen cartilage

The difference in greyscale values between fresh and frozen cartilage specimens demonstrated a decreasing trend, indicating that the intensity of cartilage differs among the two storage conditions on the examined zones. This phenomenon was remarked in high field on MRI where T2 values of the frozen cartilage specimen decreased to 12% compared to fresh specimen [31]. The storage of the cartilage specimens, particularly in freezing conditions altered its fluid content and changed the image characteristics on MRI [32,33].

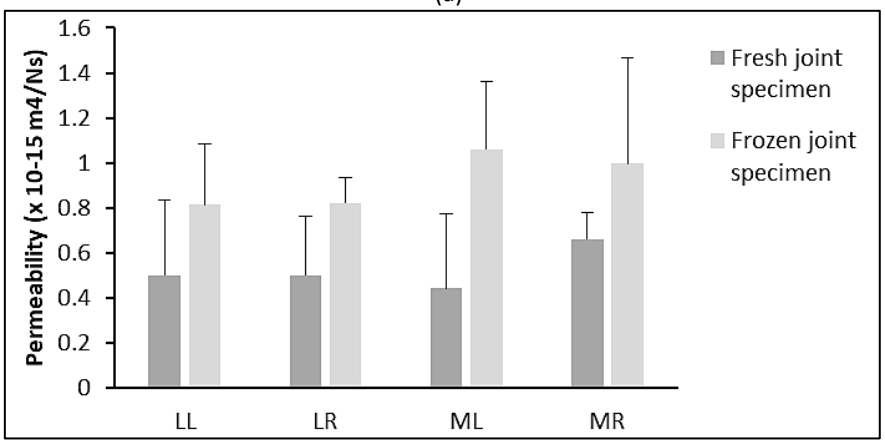
3.2 Effect of Storage Temperature on Biomechanical Properties

The experimental and computational data were integrated to characterize the cartilage biphasic properties of permeability and elastic modulus. The elastic modulus of the fresh cartilage was 1.70±0.65 MPa, while the frozen cartilage decreased to 29% at 1.20±0.47 MPa. However, the frozen storage temperature was found to be 77% significantly different on the permeability of the cartilage with $0.94\pm0.33\times10^{-15}$ m^4/Ns of frozen cartilage compared to the fresh cartilage at $0.53\pm0.25\times10^{-15}$ m^4/Ns. The biomechanical properties of the femoral head were subsequently assessed at four different quadrants known as LL, LR, ML, and MR. Similar distinct trends of permeability and elastic modulus were found compared between the fresh and frozen cartilage specimens. The elastic modulus of the frozen cartilage decreased while the permeability increased in contrast with fresh cartilage across the articular surface as presented in Figure 7.

The current outcome demonstrated the importance of preserving cartilage in biomechanical studies. The increasing demand and limited availability of viable cartilage specimens have necessitated the development and enhancement of preservation techniques that minimize the alteration of the biomechanical properties of the cartilage [31]. However, conflicting results were reported in numerous prior studies that indicated the effect of storage temperature between 4 ℃ to -80 ℃ on the biomechanical properties [10,34,35]. In particular, the freezing temperature significantly affected the permeability more than the elasticity of the tissue. This was attributed to the alteration of the extracellular structure in frozen cartilage, where the water molecules loosely packed the collagen fibrils that crystallize into ice and attract surrounding water to form convex ice lens [13]. The cooling rate disparities may also influence ice formation during freezing cartilage, leading to biomechanical behavioral changes.



(a)



(b)

Fig. 7. Biomechanical properties at 4 significant quadrants of femoral head (a) Elastic modulus (b) Permeability

3.3 Correlation of MRI Image Greyscale and Biomechanical Properties

The correlation between the MRI greyscale and biomechanical properties of the cartilage specimens for fresh and frozen conditions was examined using Linear Pearson analysis. A positive correlation was observed between the elastic modulus and MRI greyscale where a strong correlation (r=0.85) was obtained for the fresh cartilage and a moderate correlation (r=0.66) for the frozen cartilage as shown in Figure 8(a). In contrast, a negative correlation was found between the permeability and MRI greyscale in which both fresh (r =-0.51) and frozen (r=-0.56) cartilage specimens were moderately correlated as shown in Figure 8(b).

The correlation analysis for frozen cartilage specimens indicated that the storage temperature significantly affects the MRI image greyscale and biomechanical properties of the cartilage. The signal intensity of the MRI image greyscale showed a clear decreasing trend from the frozen to fresh cartilage. This is most likely due to the loss of free water molecules from the extracellular matrix component induces ice crystal formation in freezing condition [12,36]. The ice crystals can cause variations in image intensity that correspond to differences in the T2 value that were observed by numerous computational assessments [31,37]. In addition, the freezing effect on extracellular fluids in cartilage is clarified by the increased negative correlation between tissue permeability and image greyscale in frozen cartilage specimens.

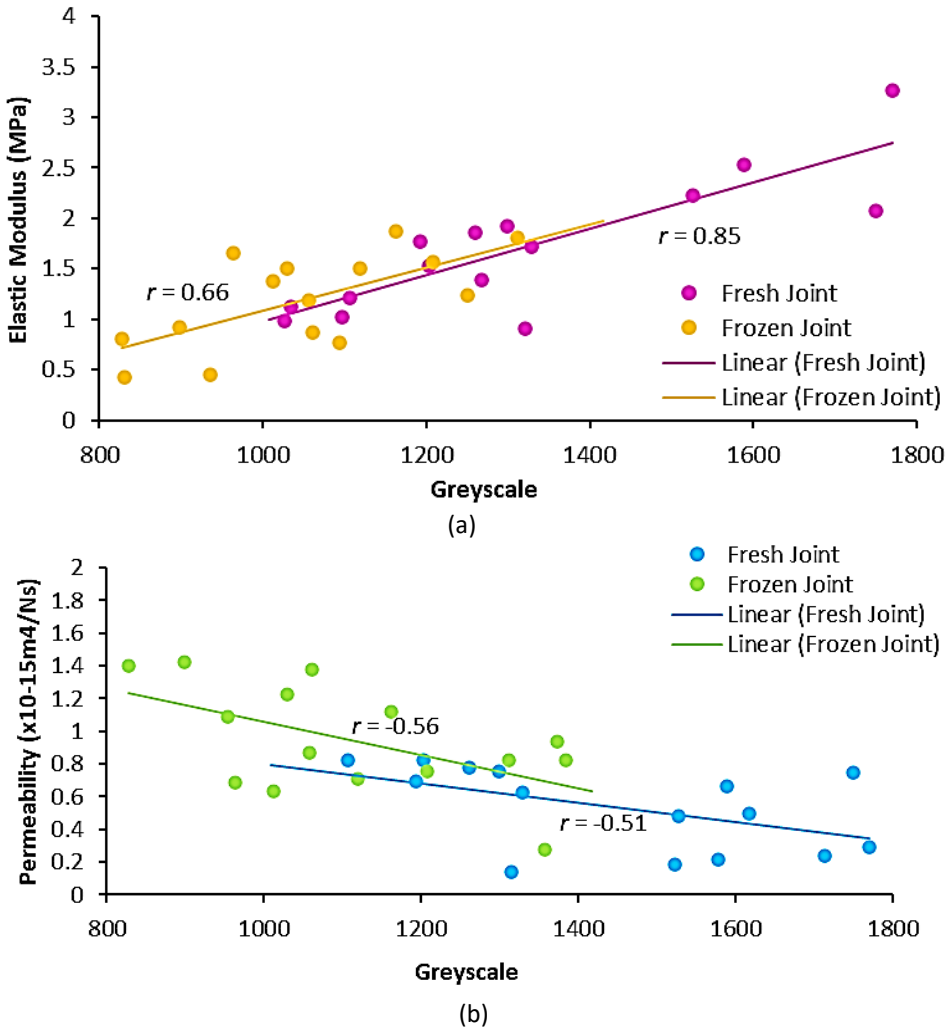


Fig. 8. Linear Pearson correlation on the cartilage greyscale and biomechanical properties (a) Elastic modulus (b) Permeability

4. Conclusions

This study indicates the importance of storage protocols to preserve the biomechanical properties and MRI images of articular cartilage. Significant differences in biomechanical properties were observed which required additional attention to the storage temperature for cartilage tissue. The permeability was more affected after a single freeze-thaw cycle compared to the elastic modulus. Although various freeze-thaw protocols were used to store the cartilage in previous studies, it is essential to identify an appropriate storage protocol to maintain the integrity of the extracellular matrix in the tissue.

Acknowledgement

This research is supported and funded by the Universiti Teknikal Malaysia Melaka (UTeM) grant scheme (PJP/2022/FKP/S01873).

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