The Influence of Delipidization Method on the Stiffness of Articular Cartilage

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Abstract

Background

Lipid depletion in articular cartilage is known to be an indication of osteoarthritic degeneration. However, the role of lipids in cartilage load-carriage is still poorly understood. In a previous study, we delipidized cartilage with chloroform which induced cell damage and cytotoxicity. In this study, we present comparative results of the biomechanical responses of articular cartilage when delipidized with milder and biocompatible solvents ethanol (C_2H_5OH) and propylene glycol ($C_3H_8O_2$).

Methods

Fours groups of bovine articular cartilage specimens (n=16/group) were subjected to compressive loading at four different strain-rates before and after delipidization in three solvents. The load-displacement curves were recorded and the corresponding stress-strain curves were plotted. The stiffness was calculated from the stress-strain curves at chosen points and compared.

Findings

Relative to normal intact articular cartilage, stiffness of delipidized cartilage, at low strain-rates (10^{-3}/s and (10^{-2}/s)), decreases by 45% on the average when rinsed with a strong solvent like chloroform, and by 20% on an average when rinsed with ethanol or propylene glycol. Stiffness increases by at least 25% at higher strain-rates (10^{-1}/s to 10^{1}/s) relative to normal intact articular cartilage. Stronger solvents are able to extract more lipids from the matrix in shorter durations but seem to induce cell damage. Prolonged exposure to mild solvents seems to stiffen the tissue inducing higher stiffness responses.

Interpretation

Milder solvents used as agents to delipidize articular cartilage do not cause cell damage and are therefore recommended for research involving articular cartilage delipidization.

Keywords: articular cartilage, lipids, rinsing methods, propylene glycol, stress attenuation, stiffness response

1. Introduction

Articular cartilage is found at the ends of articulating joints such as knees and hips, and it prevents bone-to-bone contact while performing two very important functions: (i) distribution of physiological loads acting on the joint and (ii) joint lubrication. Structurally, articular cartilage has a very complex biological character. It consists of an avascular matrix of collagen and proteoglycans (PGs) that is continuously manufactured, remodeled and replaced by the highly metabolically active chondrocytes (cell population) (Broom, 2003). Also present is a trace quantity of lipids in the intracellular matrix which are isolated in minute lacunae attached to the chondrocytes (Stockwell, 1967; Bonner et al, 1975).

Intact articular cartilage is a prerequisite for the effective physiological activity of the articulating joint. Its biomechanical integrity is regulated by the chondrocytes and determined by the mechanical properties of the collagen-proteoglycan architecture (Mow et al, 1995). It undergoes numerous age- and activity-related changes that result in joint diseases such as osteoarthritis. While the aetiology of such joint diseases is speculated to be a confluence of numerous factors, the disease manifests itself as focal cartilage degeneration (Buckwalter et al, 1997). Cartilage swelling and surface defects in the form of fibrillation leading to further erosion of the cartilage matrix along with calcification (Bank, et al, 2000). Previous studies on cartilage degeneration have examined the load-bearing characteristics of the tissue using models derived from the depletion of proteoglycans (Oloyede et al, 1994) or disruption of collagen fibres (Bank et al, 1997) in the cartilage matrix and lipid extraction (Oloyede et al, 2004).

However, also present on the surface and within the articular cartilage matrix are surface active phospholipids and neutral lipids respectively (Stockwell, 1967, Bonner et al, 1975, Pickard et al, 1998; Vecchio and Hills, et al, 1999) Consequent on their classical histochemical studies of unloaded articular cartilage Bonner et al (1975), hypothesized that lipids may have a role to play in the overall health and function of the tissue. Guerra et al, (1996) proposed that the tissue is nourished by the synovium via a semi-permeable membrane which constitutes a microscopic

overlay of surfactants on the articular surface. These layers of surface active phospholipids (SAPLs) are already established as being responsible for maintaining lubrication in the joint (Pickard et al, 1998; Vecchio and Hills, et al, 1999). Recently, lipid depletion studies have gained interest, because lipids are now acknowledged as one of the components that are compromised in osteoarthritis (Ballantine and Stachowiak, 2002).

Despite their apparent importance, there has been no quantitative data relating lipid content to the load bearing responses of articular cartilage until recently (Oloyede, et al, 2003, 2004). These studies applied the lipid extraction procedure of Hills et al (1984). We found that the major drawback with the delipidization procedure was the vacuum dessication procedure which can result in excessive tissue dehydration, thus inducing damaging strain levels which in turn, can influence any biomechanical results obtained in subsequent experiments. The method of determining the final moisture condition of the tissue by touch only is unsatisfactory and the integrity of the biomechanical results can be enhanced by finding other means of extracting lipids from the tissue's matrix. Therefore, there is a need to find alternative methods for extracting lipids from articular cartilage for scientific investigations that we can be sure is not too aggressive and with the probability of causing collateral damage to any other matrix component. In this study, we have chosen two milder rinsing agents namely propylene glycol and ethanol and compared their efficacy with that of chloroform, objectively to recommend a biocompatible lipid extracting agent for the development of accurate *in vitro* degenerated cartilage models relative to lipid loss only. Our investigation involves the bulk extraction of lipids from both the surface and the general matrix of cartilage samples, thereby extending this study beyond those carried out to investigate the consequences of surface lipid loss on lubrication. We will conduct histological examination of both normal and lipid-depleted samples to examine the relative changes to the lipid profiles in cartilage samples before and after delipidization and use these as the benchmark parameters for determining whether or not a rinsing agent might lead to collateral effect/damage in a delipidised sample, since

our aim is to identify the rinsing agent which will extract an adequate amount of lipid, while preserving the integrity of the other components of a cartilage sample.

The previous studies to map the lipid profiles in the articular cartilage matrix discussed above were purely classical, adopting the chloroform/methanol ($CHCl_3$) rinsing procedure, with none of them. addressing the possibility of (a) collateral damage as a result of the induced cytotoxicity of the strong $CHCl_3$; (b) the probably consequence of vacuum drying on the cartilage when extracting chloroform residue from samples. Consequently, in this study, we will compare the biomechanical properties of cartilage samples which have been delipidized with methods not involving the deleterious effects (a) and (b) above. Also, chloroform is not a biocompatibile solvent and should not be used to treat tissues such as articular cartilage. We will study the viability of propylene glycol ($C_3H_8O_2$) and the relatively stronger solvent, ethanol (C_2H_5OH) as rinsing agents, both of whose action are expected to be milder on cartilage than chloroform. Propylene glycol possesses *in vivo* biocompatibility (Suggs et al, 1999) and precludes vacuum desiccation which is associated with a complex dehydration-rehydration cycle. The results of this *in vitro* study will have implications for developing experimental models mimicking lipid-related consequences of osteoarthritis *in vivo*.

2. Methods

2.1 Materials

Articular cartilage specimens were obtained from the patellar grooves of 3-4 year old bovine animals (prime oxen) and stored at -20 °C until required for testing. The joint samples were thawed out in continuous running water at room temperature and preserved in 0.15M saline solution. Full thickness samples of articular cartilage were shaven off the patellar grooves and trimmed to 15x15 mm square areas. Their thickness were measured using digital Vernier calipers (DigiMax, KWB Swiss, Berne, Switzerland) at 4 different zones, the average of which was used to normalize the deformations that was used to calculate the strain for plotting the stress-strain curves. The sample

was then weighed on a digital weighing machine and its wet weight was recorded. Following this, it was glued to a stainless steel of 8mm thickness plinth using Loctite® 454 tissue glue. The stainless steel plinth with the glued cartilage was immersed in a 0.15 M saline bath and subjected to a compressive loading at four different strain-rates, on a 25kN Hounsfield testing facility. All articular cartilage specimens were subjected to a peak load of 25kN using a 10 mm diameter cylindrical stainless steel indenter, whose circular cross-sectional area was used to normalize the load to determine the stresses plotted against strain in the stress-strain data presented in this paper. The plotted values of strain were determined by dividing the measured displacement on the Hounsfield testing machine by the original thickness of each tested sample. Following deformation at a given strain-rate, the specimen was transferred into a beaker containing 0.15 M saline solution and 0.2 g/l sodium azide where it was allowed to recover for at least one hour before the next loading test. This recovery time had been previously determined as an adequate duration for the cartilage sample to recover to its original unloaded thickness (Oloyede et al, 2004). Once the four different strain-rate loading sequences had been performed on a specimen, a cynoacrylate debonder (RS Components, Birmingham, UK) was applied to the interface region between the sample and the stainless steel plinth for two minutes to dissolve the glue and debond the sample from the plinth. In a control experiment, we established that this debonding process has no consequence on the stress-strain response of articular cartilage. Each sample was then carefully wrapped in a soft tissue paper and sealed using paper tape and stored in another container containing a mixture of 0.15M saline solution and 0.2g/l sodium azide and ready for delipidization. This protocol of wrapping the sample was adopted in order to prevent osmotic curling of the sample around itself which would make subsequent tests difficult to implement because of the inherent difficulty in uncurling the tissue.

2.2 Repeatability Tests

Mechanical experiments involving multiple loading of biological specimens such as articular cartilage under different physiological and constraint conditions require that a statistical "significance level of confidence" is established by performing repeatability studies under well-

controlled conditions before any deviations can be considered as a true reflection of alterations in the tissue's condition. In order to ascertain that any variations in the observed biomechanical responses is due to the extraction of lipids, a series of tests were performed on 8 normal intact cartilage samples at four different strain-rates. The tests were repeated twice on each sample. A minimum interval of at least one hour was allowed between each loading test on a given specimen to ensure full recovery of the specimens before further loading. The strain-dependent stiffness of each sample was calculated at 0, 10, 20, 30 and 40% strain by taking the slopes of the tangents to the strain-strain curves at these points for each strain-rate.

2.3 Surfactant Rinsing and Delipidization Process

It is now well established that lipids are present both on the surface of articular cartilage as surface active phospholipids and intramatrix in intimate association with the chondrocytes. The methods of delipidization used in this study extracted indiscriminately all types of lipids from the tissue samples. This was confirmed with the sensitive weighing procedure and for establishing the weight of the samples before and after delipidization. The outcome of weighing was similar to that obtained using NMR spectroscopy (Oloyede et al, 2003), a more expensive process for determining the amount of lipid extracted.

(a) Chloroform Rinsing: In accordance with the method of Hills et al (1984), the normal intact articular cartilage specimen was removed from the saline solution and gently blotted with *kimwipe* to absorb any saline at its surface and then transferred into a 100-ml glass reagent bottle containing 80-ml of 2:1 chloroform/methanol. This process is not expected to have any adverse effect on the tissue. The reagent bottle with the cartilage specimen was then placed on an agitator tray to gently agitate the specimen in the chloroform environment at room temperature for 30 minutes. The specimen was removed from the reagent bottle and its surface was again blotted with *kimwipe* sheet before transferring it to a desiccator where the $CHCl_3$ was evaporated from the cartilage in vacuum for one hour. This one hour duration was obtained as optimum through repeated trail-and-error experiments during which the specimen was touched at regular intervals of 10 minutes to determine

its suppleness or state of dehydration. Beyond the one hour dessication, the specimen was found to rapidly dehydrate and assume a leathery condition relative to touch. After the one hour, it was removed from the desiccator and allowed to rehydrate in 0.15M saline solution to minimize its drying or shrinkage of the specimen and hence any concomitant pre-strain. In this type of process, it is practically impossible to avoid a level of dehydration, which might result in prestraining the specimen, but we believe that this touch-based assessment helped minimize this effect. After a recovery period of one hour in saline solution, the sample was glued onto the stainless steel plinth and then returned to saline to recover for another hour, after which the sample was again loaded in compression with intermittent recovery periods as mentioned earlier.

(b) Propylene Glycol and Ethanol Rinsing: Four groups of normal articular cartilage specimens (n=16/group) were rinsed with propylene glycol and ethanol. Two groups were rinsed for 30 minutes while the other two were rinsed overnight (15 hours). Each sample was removed from the saline solution and gently blotted with *kimwipe* to absorb any excess saline on their surfaces and then transferred into 100-ml glass reagent bottles containing 80-ml ethanol (C_2H_5OH) and propylene glycol. Two of the reagent bottles with the cartilage specimens were then placed on an agitator tray to gently shake the specimen in the ethanol environment at room temperature for 30 minutes, while the other two bottles were left overnight for 15 hours. After the delipidization process, all the samples were once again subjected to the compressive loading sequence outlined in section 2.1 with intermittent full recovery between loads. All samples were weighed with a digital weighing scale after delipidization and their wet weights were recorded and compared with those of their normal counterparts.

2.4 Lipid Extraction and Histology

Histology studies were conducted on articular cartilage samples to evaluate the lipid profiles of normal and delipidized samples.

2.4.1 Sample Preparation

A 4x4 mm cube of cartilage-on-bone sample was first obtained from the lateral plane of the patellar groove. The cube was then sliced into four smaller (1x1) mm samples. One sample was treated as the control group while each of the other three was rinsed in a particular solvent, viz., chloroform, ethanol and propylene glycol for 30 minutes. All the four samples were then decalcified for a period of 28 days with periodic x-ray evaluation to estimate the extent of decalcification. After decalcification, each sample was cryo-frozen in liquid ammonia and several sections of formal-calcium fixed slices of the tissues were prepared for microscopic examination. A total of 20 sections were prepared (5/sample) and the lipids were localized by coloration with Oil Red O according to the staining procedure described in Bonner et al (1975). An attempt was made to assess the amount of lipids contained in the intra-cellular matrix of the slices obtained from normal and all delipidized samples. The cell count was set at 100 in each case (slice) so that the lipid content could be expressed as percentage, with only the lipid globules that measured 1 μm or greater in diameter counted based on the assumption that all the lipid globules were spherical. Using these cell counts, the mean values of the lipid content per cell was estimated for all samples.

2.5 Statistical Analysis

Repeated Measures (RMANOVA) statistical analysis was performed on the data set using the statistical package SPSS 12.0 for Windows. This analysis was to determine how significant a particular delipidization method was on articular cartilage stiffness. Stiffness was set as a dependent variable while cartilage thickness, compressive strain, cartilage types (normal and delipidized) and the method of delipidization were considered as independent variables. RMNOVA at the 95% confidence level, showed that the method of delipidization has a significant influence on the dependent parameter (stiffness) with $P = 0.012$, n = 16 for chloroform, $P = 0.032$, n = 32 for propylene glycol and $P = 0.023$, n = 32 for ethanol.

3. Results

3.1 Lipid Extraction

Figure 1 shows the histology of the cartilage matrices post delipidization. The samples were microtoned and stained with Oil Red O. Figure 1b shows the complete absence of any red color in the histology section which indicates that a high level of delipidization occurred when using chloroform as the rinsing agent. Figure 1c shows a close up of Figure 1b which indicates some level of microstructural cell-level changes while these were not perceivable in the histology of cartilage that was delipidized with the milder agents. Figures 1(d) and Figure 1(e) correspond to the histology of cartilage rinsed with ethanol and propylene glycol respectively and Figure 1(f) is an enlargement of a section of Figure 1(e). Unlike Figure 1(b), a trace presence of lipids can be noticed in these slides indicating the mildness of the rinsing agents. Table 1 presents the average wet weights of normal and delipidized cartilage samples which were used to evaluate the average percentage lipid loss in the samples after treatment with chloroform, ethanol and propylene glycol. It can be seen that the percentage reduction in lipids for chloroform after exposure of normal intact tissue for only 30 minutes is of the same order as the amount extracted with exposure to ethanol and propylene glycol for 15 hrs. Also, it is revealed in this table that very small trace amounts of lipids were extracted using the milder rinsing agents, namely, 0.64% and 0.32% for ethanol and propylene glycol respectively after the shorter duration of exposure of 30 minutes.

3.2 Stiffness Variation in Repeatability Tests

The analysis of the repeatability tests on normal articular cartilage showed that at 10^{-3}/s strain-rate, the stiffness varied by less than 1% over the 0-40% strain region, by less than 1.5% at a strain-rate of 10^{-2}/s and 10^{-1}/s, and by about 1.8% at the highest strain-rate of 10^{1}/s. We argue that these marginal variations in stiffness values are negligible; thereby rendering any observed changes between normal and delipidized samples in subsequent experiments the consequence of reduction in lipid contents.

3.2.1 Influence of vacuum desiccation on stiffness

Four articular cartilage samples were subjected to compression and delipidized after recovery in 0.15M saline solution. After delipidization they were transferred into a desiccator and subjected to vacuum to remove any excess solvent from their matrices before subjecting them to the same compressive loading cycles of load-recovery-delipidize-recovery-load. Our results show that the stiffness of vacuum desiccated samples was on an average 15% greater than those that were not subjected to the procedure.

3.3 Stress-strain responses

Figure 2 presents the comparison of the representative stress-strain responses of normal and delipidized articular cartilage when delipidization had been carried out using chloroform. This pattern is consistent with our earlier study in which chloroform had been applied as a rinsing agent (Oloyede, et al, 2003). Figure 3 compares the representative responses of normal and delipidized cartilage where propylene glycol was used to rinse the samples for 30 minutes while Figure 4 shows the responses for ethanol rinsed cartilage samples. It can be seen that the stress at a given strain of delipidized cartilage is lower than that of its normal counterpart at low strain-rates of 10^{-3}/s and 10^{-2}/s and higher at high strain-rates of 10^{-1}/s and 10^{1}/s. The stiffness under different loading rates of normal and delipidized articular cartilage specimens were calculated at three strains of 0%, 10%, 20% and 40% using a MATLAB program from the original stress-strain curves obtained for each experiment by estimating the slope of the stress-strain curves, at the strains mentioned above,

Table 2 presents the values of the stiffness for both normal and cartilage that were dilipidized with the three rinsing agents evaluated at different loading velocities. At the lower strain-rates of 10^{-3}/s to 10^{-2}/s, the samples rinsed with propylene glycol exhibited between 10 and 35% lower stiffness in comparison with to normal counterparts while at the same loading rate, the stiffness of the cartilage samples rinsed with ethanol was lower by 45-50%, with the stiffness values estimated in the strain range of 0 to 40%, relative to that of the normal intact samples. Comparatively, the stiffness of samples rinsed with chloroform dropped by 70-90% at this low strain rate of 10^{-3}/s in the same

strain range of 0 to 40%. At the higher rate of loading of 10-2/s, the relative decreases in the stiffness of delipidised relative to that of the normal sample were 5-10%, 30-45% and 60-70% over 0 to 40% strain for propylene glycol, ethanol, and chloroform rinsing respectively. The delipidised matrices exhibited an increase in stiffness relative to their normal intact counterparts from the higher loading rate of 10^{-1}/s, with increases of 5-25%, 20-35% and 25-30% in the deformation range of 0 to 40% strain for propylene glycol, ethanol and chloroform rinsing respectively. This pattern of increase in the stiffness of the delipidised compared to that of the normal intact matrix continued at the higher loading rate of 10^{1}/s. At this speed of loading and in the strain range 0-40%, propylene glycol rinsed samples exhibited higher stiffness of 5-27%, ethanol-rinsed 25-36% and chloroform-rinsed 30-50% relative to their normal intact counterparts.

In order to complete this study, the influence of prolonged exposure of cartilage to milder rinsing agents was investigated. 16 samples each were treated with propylene glycol and ethanol for 15 hours. In both cases, the reversal of stiffness was observed at 10^{-2}/s strain as against that seen in the case of 30 minute rinsing where the stiffness of the delipidized cartilage samples became higher than the normal samples at the higher strain-rate of 10^{-1}/s, as can be seen in Table 2.

4. Discussion

In this study, we compared the performance of three solvents that could be used to delipidize articular cartilage to determine a safe rinsing procedure. The histology of cartilage sections from the three different rinsing methods indicates that certain effects other than tissue dehydration may occur when using strong rinsing agents such as chloroform. Further, it is still unclear if the use of stronger agents comprises the cell walls, as indicated in Figure 1(c), and the cell environment as lipids are found in minute lacunae that are attached to the cells or if it is the vacuum dessication that influences the hydro-capsule surrounding the cells causing damage to the cell walls. While we do not expect any microscopic damage to cells to influence the biomechanical behaviour of the loaded

316 cartilage matrix, it is our opinion that any effects other than the removal of lipids by any agent is an
317 unacceptable contribution to the obtained biomechanical data and the subsequent comparative data
318 analysis, especially as we are unable quantify such collateral effects absolutely for different
319 samples.
320 On another note, it is probable that any agent that influences other components of the matrix in
321 combination with delipidization might also adversely affect the collagen-proteoglycan framework
322 which governs the load-induced biomechanical responses of the cartilage. However, it is not the
323 intention in this present study to measure either the collagen disruption or proteoglycan loss.
324 Compressive loading tests at different strain-rates which model physiological loading conditions
325 were carried out to determine how the load-carriage of the tissue is influenced both by the extent
326 and method of delipidization. Our results indicate that irrespective of the type of delipidization
327 method, the extraction of lipids from the cartilage matrix results in apparent embrittlement of the
328 tissue leading to a concomitant increase in its stiffness relative to the normal intact tissue especially
329 at high rates of loading of 10^{-1}/s to 10^{1}/s, with an increase, relative to the normal samples of
330 between 20 and 30% on an average. This situation is reversed with the stiffness of the lipid depleted
331 samples being lower by between 10 and 30% relative to the normal, at lower rates of loading of 10^{-3}/s to 10^{-2}/s. This dramatic pattern exhibited by the stiffness at low and high strain rates relative to
333 the normal sample appears anomalous with respect to what has been customarily observed for
334 normal intact cartilage where the stiffness continuously increased with rising strain rate (Oloyede et
335 al, 1992). This phenomenon is not fully understood at this stage. However, we hypothesize that the
336 pattern is indicative of the non-linear interaction between the fluid (which is unaffected by the
337 process of delipidization), the solid skeleton (which is stiffened) and the speed of loading.
338 Consequently, we argue that at the lower strain rates the fluid's action is more predominant in the
339 load processing of the delipidised samples as a consequence of the slower rate of deformation of the
340 stiffened solid, relative to the solid in the normal intact matrix. In this regime of behaviour the
341 overall response of the matrix is poroelastic. On the other hand, it seems that at the higher rates of

loading the stiffened solid, due to its slower deformation, resisted most of the load resulting in significant reduction in fluid loss leading to a viscohyperelastic deformation of this solid component, and an overall matrix response that can be described as poro-viscohyperelastic. Given that joint function is dependent on lubrication (with fluid flow implication – weeping lubrication) [McCutchen,1959] and deformation of cartilage, the bifurcation of the stiffness into distinct patterns at high and lower strain-rate may have significant consequences for physiological load bearing. This is because the much higher stiffening relative to the normal intact material at high rates of loading can be argued to be indicative of a higher susceptibility to inadequate load bearing deformation accompanied by lower volume of exuded water to facilitate lubrication, thereby, leading to an increased wear rate and cartilage damage.

Our determination of suitability of a rinsing agent has been based on the ease with which the cartilage could be delipidized with minimum or no collateral damage to the tissue's integrity. Essentially, we found that a 30-minute rinsing of cartilage using milder rinsing agents, relative to the highly aggressive chloroform, could also produce substantial lipid reduction in a cartilage matrix. However, milder agents, namely propylene glycol and ethanol are not as effective as the stronger agent over short rinsing periods where the milder agents extracted a much smaller amount of lipid in comparison to that extracted by chloroform for the same period as seen in Table 1; but the use of milder and biocompatible rinsing agents preclude the need for additional treatments such as vaccum dessication and rehydration and no chance of inducing any cytotoxicity in the tissue. Based on the assessment of Wuthier (1968), the estimated lipid content in bovine articular cartilage is approximately 7%. If this value was equated to the maximum possible in any cartilage (i.e.100%), then the percentages of the lipids extracted by each of the rinsing agents relative to this maximum can be estimated to be 55%, 5% and 10% for chloroform, propylene glycol and ethanol respectively when the tissue is exposed the agents for 30 minutes. When the duration of rinsing was increased to 15 hrs, the amount of lipid extracted from the matrix relative to the total quantity contained in the normal sample was found to be 55% and 60% for propylene glycol and ethanol

respectively. It should be noted that high lipid extraction rates are the most desirable conditions for studying the effects of osteoarthritis on cartilage since the tissue could lose up to 95% of its lipids depending on the severity of the disease. This situation is difficult to achieve with the milder rinsing agents over a short period of time, also there is a distinct possibility that the integrity, relative to subsequent loading test results, of any tissue samples treated in these milder rinsing agents over prolonged durations cannot be ascertained. This situation inevitably requires further research.

5. Conclusions

While this study resolves the choice of rinsing agents for delipidizing articular cartilage without collateral damage to its cells and arguably other tissue components, it still leaves a wide gap with respect to the extraction of the levels of lipids known to occur in osteoarthritis in a time duration that will preserve tissue integrity.

6. Contributors

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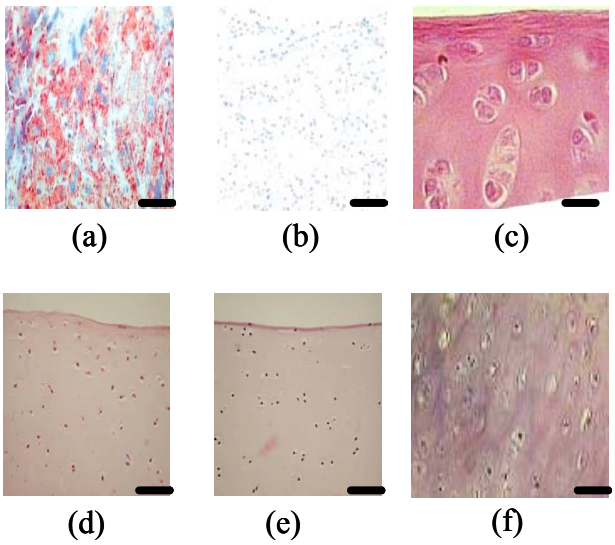

Figure 1: Histology of Normal and Delipidized Articular Cartilage

(a) Normal Cartilage stained with Oil Red O, (b) Cartilage delipidized with $CHCl_3$

(c) Enhancement of slide (b) showing cell changes due to $CHCl_3$ rinsing,

(d) Cartilage delipidized with Ethanol, (e) Cartilage delipidized with Propylene Glycol

(f) Enhancement of slide (e)

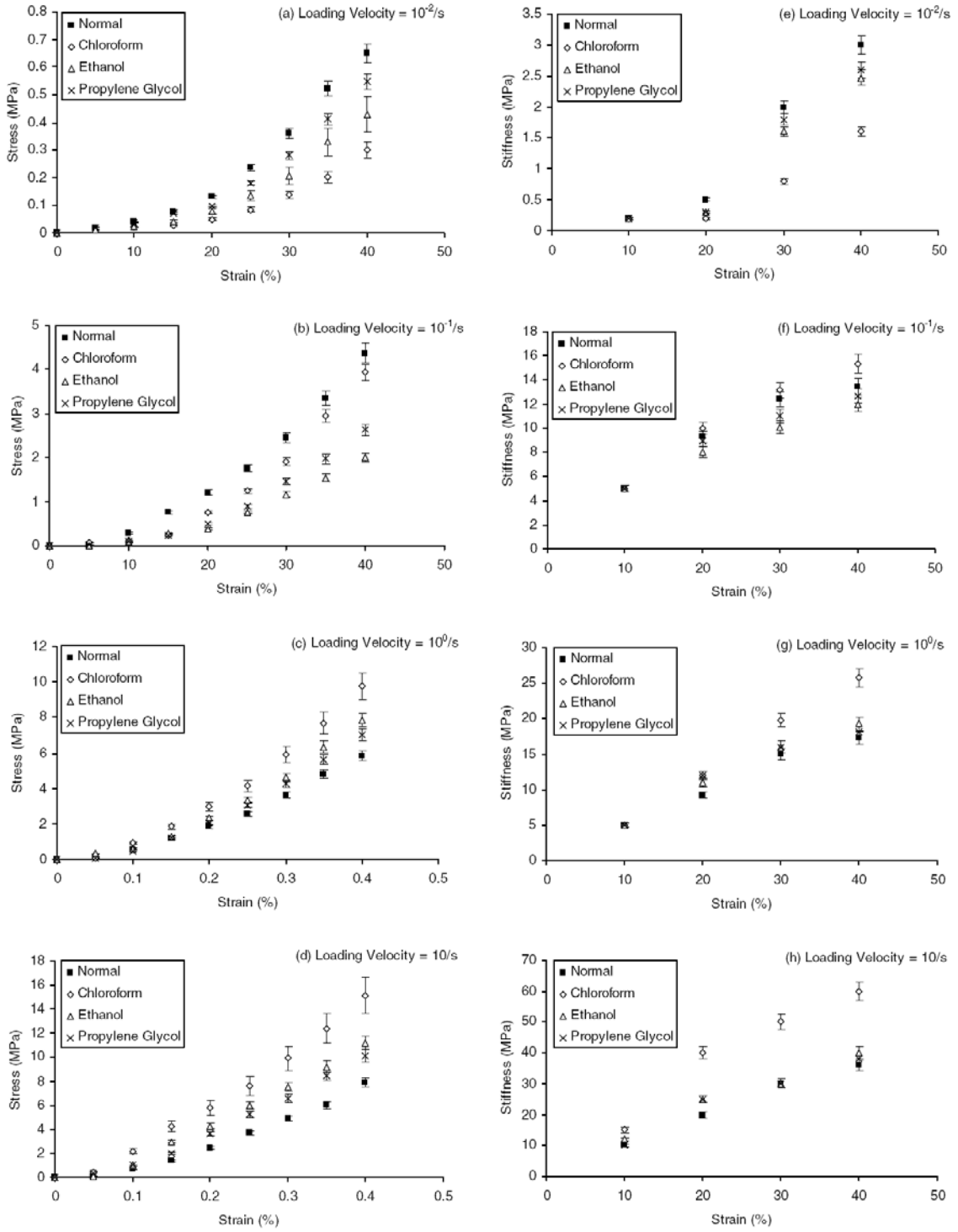

Figure 2: Stress-Strain and Stiffness Curves of Normal and Delipidized Articular Cartilage at different Loading Velocities

Table 1: Relative Changes in W/W and Lipid Content in Delipidized Articular Cartilage

Typical Thickness	Normal	Delipidized				
1.4mm		Chloroform ($CHCl_3$)	Ethanol (C_2H_5OH)		Propylene Glycol ($C_3H_8O_2$)	
		30 minutes	30 minutes	12 hours	30 minutes	12 hours
Wet weight (g)	0.32	0.311	0.315	0.309	0.316	0.310
Lipid Content (%)	3.40	2.600	1.450	3.200	1.000	3.000

Table 2: Stiffness of Delipidized Articular cartilage

Rinsing Agent (30minutes)	**Thickness** (mm)	**Stiffness Relative to Normal (%)**			
		10^{-3}/s	10^{-2}/s	10^{-1}/s	10^{1}/s
Chloroform ($CHCl_3$)	1.1-1.6	35~40↓	20~30↓	20~25↑	25~35↑
Ethanol (C_2H_5OH)	1.1-1.6	15~20↓	15~35↓	10~20↑	10~20↑
Propylene Glycol ($C_3H_8O_2$)	1.1-1.6	5~10↓	10~15↓	5~15↑	5~10↑
12 Hours					
Ethanol (C_2H_5OH)	1.3-1.5	30~40↓	20~35↑	15~25↑	10~25↑
Propylene Glycol ($C_3H_8O_2$)	1.3-1.5	15~20↓	15~25↑	20~35↑	20~30↑

↓ - Delipidized cartilage is softer than normal counterpart
↑ - Delipidized cartilage is stiffer than normal counterpart