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RELIABILITY AND REPRODUCIBILITY OF 2-DIMENSIONAL ELECTROPHORESIS IN DETERMINING THE STANDARD PROTEIN PROFILE OF HEALTHY RABBIT CHONDROCYTES

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ABSTRACT
Differential proteome analysis is important to determine the differences of protein expressed between two or more sample conditions. An analytical strategy to overcome variation between samples was also needed to ensure that future extrapolated data would not result in misleading conclusions. Therefore, in this study, we determined to measure the reproducibility of the observed spot patterns among technical replicates as well as biological replicates in the proteome of rabbit chondrocytes. Two-dimensional electrophoresis (2-DE) of chondrocyte intracellular protein was carried out in replicates from related and unrelated-sources of healthy adult rabbits. Following analyses (n=9), average matching intra-sample (within the same rabbit) gel patterns was 52.89% ± 8.18% (SD) in relation to spot volume percentage. Conversely, the average matching of the inter-sample (different rabbit) gel patterns was 32.20% ± 11.45% (SD). We can therefore conclude that the proteome mapping established using adult rabbit chondrocytes proved reliable and highly accurate in determining the presence of high abundance proteins.

Keywords: Articular cartilage; chondrocytes; rabbit; proteome.

1. INTRODUCTION
Articular cartilage consists of 3 major components which include chondrocytes (articular cartilage cells), extracellular matrix (ECM) and water [1].Collagen and proteoglycans are the two principle components of cartilage extracellular matrix; responsible for maintaining tissue tensile strength is consistent volume and shape. Type II collagen forms the

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predominant type of collagen in articular cartilage and forms the basic fibrillar structure of the matrix. Proteoglycan is found primarily as large aggregating proteoglycans (aggrecan and versican) which make up 50-85% of proteoglycan content cartilage while the large non-aggregating forms make up the remaining 10-40% of total proteoglycan [2]. As articular cartilage is avascular and aneural ECM synthesis and regulation are controlled within the tissue by the resident chondrocytes.

Chondrocytes are intimately surrounded by extracellular matrix and have limited (if not completely absent) cell-cell contact and thus possess a restricted ability to undergo replication [3]. However, these cells are largely responsible for maintaining cartilage homeostasis involving the synthesis of matrix components; the incorporation and organization of these components into the matrix; and degradation as well as resorption of the matrix. Thus, any disruption of this homeostatic balance results in poor extracellular matrix (ECM) remodelling, or even degradation of that matrix (which can eventually lead to osteoarthritis). Therefore, to understand normal cartilage cellular function, it is important to fully understand chondrocyte metabolism and proteins that are responsible in ECM regulation. This in turn will assist in future research which not only aims to repair diseased cartilage but also determine abnormal changes by comparing results attained from targeted samples to established normal results.

Management of articular cartilage injury has become a major healthcare problem contributing to rising financial burden globally. Irreversible degradation of cartilage matrix is a major feature in osteoarthritis [4] and rheumatoid arthritis (RA), eventually leading to a loss of joint function and thus severe disability [5]. Little is known about the molecular mechanisms involved in regulating cartilage destruction and regeneration in the diseased state [6]. However, it has been postulated that anabolic/catabolic imbalances occur in chondrocytes and results to matrix homeostasis, possibly related to destruction of homeostasis-regulating MAP kinase activity [7].The MAP kinase subtypes expressed in articular chondrocytes are thought to be responsible for multiple chondrocyte functions including differentiation, apoptosis, inflammatory responses and activation of matrix metalloproteinases. Although some studies have suggested these homeostatic imbalances do occur [8,9,10], clear evidences are still lack. Current biotechnological techniques in proteomic analysis, global protein detection and comparison have offer improved prospects of understanding these mechanisms. Much current research has focused on the analysis of proteins secreted by human chondrocytes using 2D-PAGE or other proteomic technologies with new findings on the proteins expressed being updated constantly. [11, 12, 13, 14, 15]

However, although these findings have attempted to elucidate the imbalances that occur in cartilage metabolism, reliability of the results published in the literature have been a major concern. Four major areas of concern were identified as possible sources of inconsistencies including experimental design, analysis of protein abundance data, confidence in protein identification by mass spectrometry and incomplete analysis [16]. It is therefore important that any studies using proteomic analysis to determine normal protein distribution of cells need to establish the reliability of these techniques, providing a level of confidence with regard to results and research data.

The objective of this present study was to establish a standard proteome profile of chondrocytes isolated from rabbit joints by focusing on the experimental design, reproducibility and reliability of the proteome map produced.

The role and importance of experimental design has been described for transcriptomics but less frequently for proteomics [17]. Good experimental design limits systematic errors, improves precision of subsequent statistical tests and thus reduces the number of false positives.

Differential proteome analysis of spots is generally handled by using commercial software packages that propose statistical tools to assist in concluding the significance of variation. This study is of importance to rationalize the significance of proteins being expressed and to also highlight the imperfections of gels, spots mismatches and other artefacts of 2-DE gels.

Analytical incompleteness is encountered in proteomics, especially in missing spots (missing data) on one or more gels coming from the same series. The problems resulting in these missing data on 2-DE gels are generally due to experimental problems and must be taken into consideration. Therefore, the establishment of the proteome map of

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chondrocytes by a systematic differential analysis strategy represents an important step in the utilization of proteomic techniques in tissue engineering for the treatment of damaged articular cartilage.

2. MATERIALS AND METHODS

2.1 Primary Cell Culture

Approval to harvest and culture rabbit articular cartilage was granted by the Animal Ethics Committee of University of Malaya [Reference no.: OS/06/07/2008/TKZ/A(R)]. Briefly, articular cartilage biopsies freshly harvested from rabbits were first washed with PBS and minced manually utilizing sterile forceps and a scalpel. The finely minced tissue fragments were then transferred into 15 ml centrifuge tubes and washed with PBS (10 ml) with 2% antibiotic-antimycotic in a rotating 37°C incubator for 10 minutes at 250rpm. The tissue fragments were then enzymatically digested with 10 ml of type II collagenase solution (0.6%) and incubated in the same condition (37°C incubator for 10 minutes at 250rpm) overnight. Subsequently, the digested tissue was transferred into the growth medium (DMEM/Ham's F-12 supplemented with 10% FBS and 25 µg/ml ascorbic acid) in a 75cm^2 culture flask with vented cap. The culture was incubated in an orbital shaker incubator at 5% CO2, 37°C and 95% humidity, with the culture medium changed every 2-3 days until 70-80% of cell confluence is achieved. Confluent chondrocyte cultures were subsequently subcultured (P1 and P2) for further analysis. Considering that chondrocytes are easily dedifferentiated when cells passaged as monolayer [18] in every experiments in this current study, chondrocytes utilised were grown at passage 0 (P0) to passage 2 (P2) (limited to early passages).

2.2 2-dimensional Gel Electrophoresis (2-DE)

Cells cultures were harvested and lysed for protein extraction by lysis buffer. The extracted protein was aliquoted and kept at -80°C for protein quantification and isoelectric focusing (IEF).

The protein sample clean-up using the 2-D Clean Up kit (GE Healthcare Biosciences, Sweden) was carried out according to the manufacturer's procedure to remove all salts in the sample which would disrupt the IEF. The cleaned sample (100µg) was dissolved in a rehydration buffer (450 µl; volume required to fully rehydrate a 24 cm IPG drystrip) and left for 5 minutes at room temperature. The sample mixture was loaded into the slot of the re-swelling tray and a 24 cm dry-strip with pH range from 3 to 10 was carefully lowered with the gel side down onto the sample mixture. Dry-strip cover fluid was loaded over the dry-strip and into a sample slot until it was full to minimize evaporation and urea crystallization. The IPG dry-strips were left to stand for 18 hours at room temperature to ensure complete rehydration and uptake of sample.

IEF was performed using an Ettan IPGphor™ system (GE Healthcare Biosciences, Sweden). The rehydrated strips were placed in the IPG strip holder with the gel facing the electrodes which were covered with moist filter paper. The strips were then soaked with cover fluid to ensure good thermal contact. The IEF running conditions were set according to optimize conditions (step 1 at 500V for 1 hour, step 2 at 1000V for 1 hour and step 3 at 8000V for 6 hours).

The second dimension run was performed using a constant current (120W). Electrophoresis was stopped once the dye front was approximately 1 cm from the bottom of the gel.

The 2-DE gels were developed by double-staining procedures according to the method. The gel was stained with hot coomassie blue prior to silver nitrate staining as described previously [19] with modifications. Proteins appeared as spots after the staining on the gel.

2.3 Spots Analysis

Spots analysis was performed using Image Master 2D Platinum Image software version 5.0 (GE Healthcare Biosciences, Sweden). Prior to automated spot detection by the software, 4 distinct spots found at similar locations in replicate gels could be determined as consistent as they were observed in all replicates and labeled as landmarks. These spots served as

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the references within the gel replicates for the automatic matching software. Automatic spot detection was performed with default parameters, whereas spot editing and removal of artifacts were done manually. Spots were analyzed in terms of percentage volume contribution, which referred to the volume of protein taken against the total spot volume of all proteins including unresolved peptides in each gel.

2.4 Mass-Spectrometry and Identification of Spots

Protein identification was performed using MALDI-ToF mass spectrometry in Genome Research Institute, Hong Kong. Spots were excised in duplicate and transferred to 1.5 ml siliconized eppendorf tubes for reproducibility assessment prior to in-gel digestion with trypsin. The proteins were identified by MALDI-ToF MS on the basis of peptide mass matching. The monoisotopic peptide mass fingerprinting (PMF) data obtained from MALDI-ToF MS were analyzed for protein identification against NCBInr and SWISS-PROT databases.

2.5 Experimental Replication

When investigating the proteome of cells, the subjected variability must be taken into consideration. The first source of variability involves that of biological variations. Since primary cultures were utilized in this study, biological variability could be attributed to batch-to-batch differences. Such differences occur as although general proteins may be present in all individuals, certain specific proteins may vary between them.

The second source of variability covers technical variations such as cell preparation and protein solubilization prior to separation by 2-DE. This variation in theory is assumed to be attributed to the inconsistencies that occur during the process of conducting the experiments. Considering that the cells are attained from the same source for the replicates, it is safe to make an assumption that there are no variations in the protein expressed in the samples tested.

In order to determine such variations, the number of replications was systematically set to compare the results between samples as well as between different individuals (Figure 1).

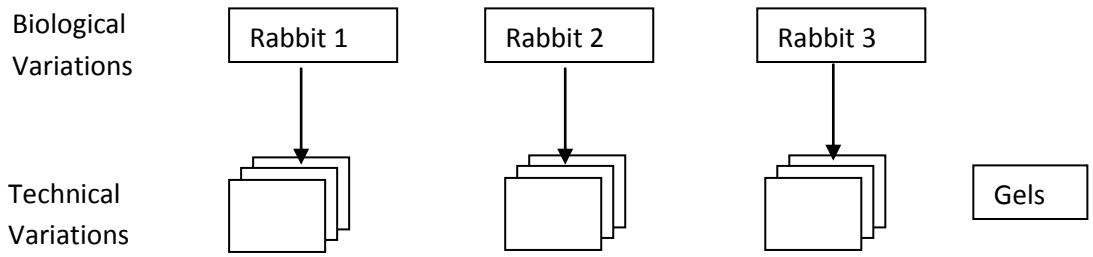

Fig 1: Schematic diagram showing the experimental design for reliability and reproducibility study using replications to overcome variability. In this experimental design, three replications (n=3) of rabbits were used to limit the biological variances and three replications (n=3) were used to limit technical variations.

2.6 Statistical Analysis

In this experiment, gel was established in triplicates (n=3) from one rabbit and total of three rabbits (N=3) were used in this study. Determination of variance was carried out by matching percentage (%) of the spots in the replicate gels inter and intra-species. Mean values of the percentage were used for our analysis on variance determination. All results were calculated and presented as mean and standard deviations (SD).

3. RESULTS

Protein concentration in the cell lysate was determined by the Bradford method[20] which plots the relationship between protein concentration and spots detected by 2-dimentional electrophoresis. It was noted that protein spots

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expressed/observed by silver nitrate staining gradually increased as protein loading for the 1-dimensional electrophoresis increased. However, the number of spots remained constant at 100 µg and 150 µg, shown as a plateau state in the scatter plot (Figure 2). 100 µg was taken as the optimal concentration for 2-dimentional electrophoresis and used to establish the standard protein profile for the chondrocyte proteome. Spots were visualized by silver nitrate staining and detected using 2D Platinum Image Master software ver. 5 (Figure 3).

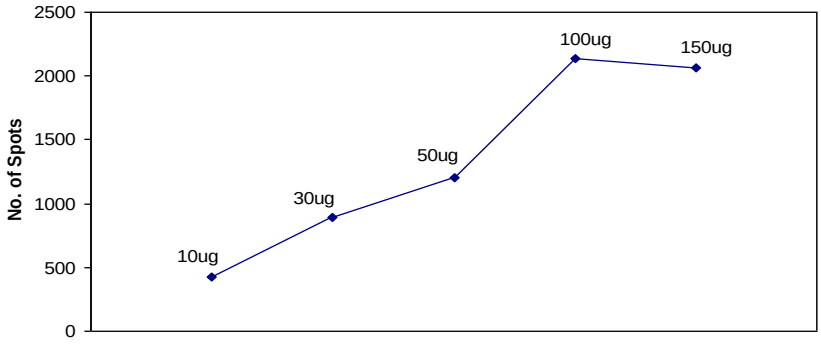

Fig 2: Scatter plot showing the effect of increased protein loading on the quantity/number of spots expressed on the protein expression profile. Protein extracted from chondrocyte lysate was rehydrated into IPG strips at 10, 30, 50, 100 and 150 µg protein concentrations prior to 2-dimensional electrophoresis. The number of protein spots visible on the gel after silver nitrate staining was gradually increased as the amount of protein loaded for the first dimensional electrophoresis increased until 100µg. Higher protein concentrations did not show any changes in the protein spots visible.

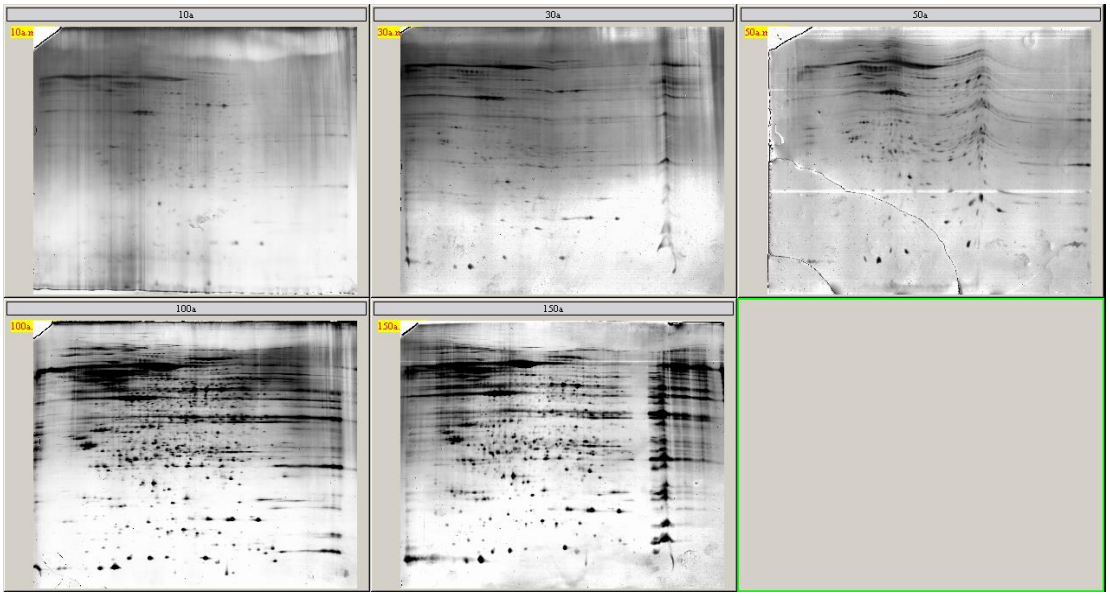

Fig 3: Determination of an optimal protein loading concentration for first dimensional electrophoresis. Protein extracted from chondrocyte lysate was rehydrated into IPG strips at 10, 30, 50, 100 and 150 µg (gel a, b, c, d and e respectively) protein concentrations. The number of protein spots visible on the gel after silver nitrate staining was analyzed by 2D Platinum Image master software.

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3.1 Spots Analysis

For 2-DE analysis, all experiments were carried out in triplicates (inter-samples and intra-samples). Prior to further analysis of gel variability, each pair of spots from gel replicates identified by the Image Master Software through the matching analysis was critically examined (Figure 4). The spots volume percentages between gel replicates were also determined. The distribution of spot volume percentage scatter plot shows a correlation coefficient (r^2) of more than 0.8 (Figure 5).

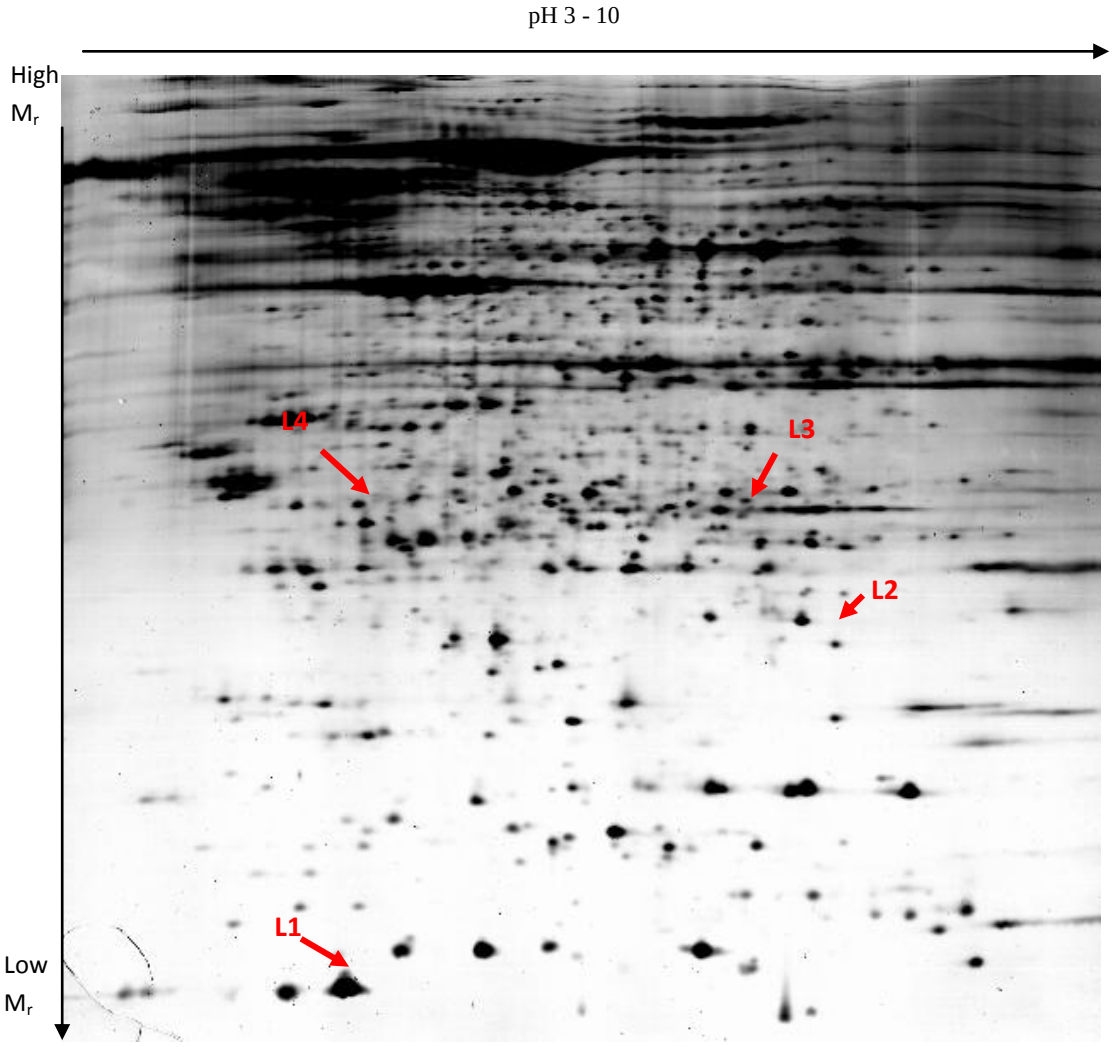

Fig 4: Representative two-dimensional electrophoresis map of cell culture extracts from rabbit articular cartilage chondrocytes (pH 3-10, 12.5% acrylamide SDS-PAGE, silver nitrate staining). Consistent spots (L1, L2, L3 and L4) have been observed in all replicates and served as landmark for gel matching analysis.

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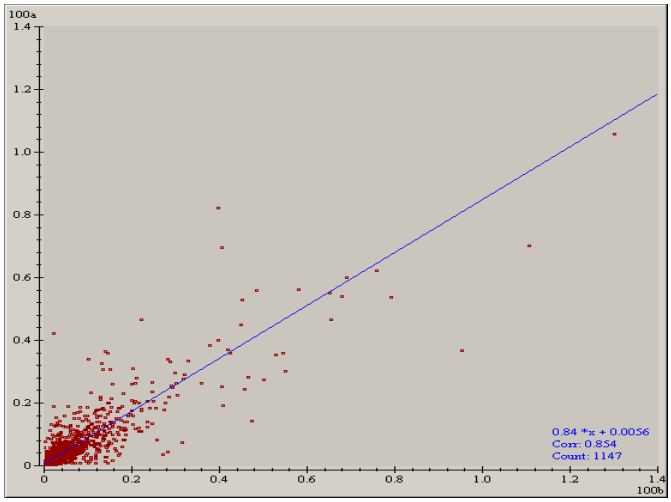

Fig 5: Scatter plot showing the spots correlation between 2 replicates gels (Gel 1 and Gel 2). The relationship on spot percentage volume between gel replicates was also established automatically by the 2D Platinum Image Master software after the matching analysis. In all plots, correlations between spots are more than 0.8. In general, all the replicates are highly correlated with all paired values are closed to the best-fit line with respect to the reference gel.

3.2 Biological Variance

In determining the biological variance in this study, three rabbits were used as replication representatives. Spots in each gel (n=9), representing samples collected from three different rabbits, were detected using the 2D Platinum Image Master software (ver. 5) and matched automatically using the selected landmarks (Figure 6). The results revealed that, spots matching between gel replicates from inter-samples (n=3) preparation were less than 50% (Table 1). This large variance in sample replicates from different entity can be attributed to the sample heterogeneity.

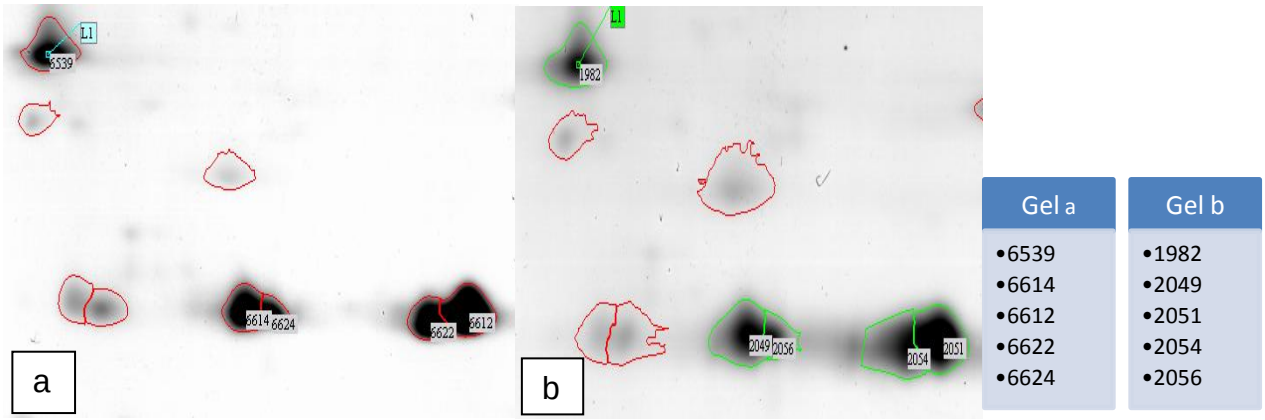

Fig 6: The spot pairs of the replicate gels were critically examined prior to establishment of the standard proteome of chondrocytes. The above data shows representative pairs of spots from replicate gels (Gel a and Gel b) from the gel replicates.

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Table 1. The matching percentage (%) between gels produced from chondrocytes of three different rabbits (rabbit 1, 2 and 3) and respective correlation (r^2) in the inter-sample variation study (n=3; N-3)

		Rabbit 1						***Rabbit 2***					
		Gel 1		*Gel 2*		*Gel 3*		*Gel 1*		*Gel 2*		*Gel 3*	
		%	*r^2*	*%*	*r^2*	*%*	*r^2*	*%*	*r^2*	*%*	*r^2*	*%*	*r^2*
	Gel 1	45.6	0.76	46.6	0.75	30.2	0.71						
Rabbit 2	*Gel 2*	47.4	0.71	42.6	0.72	39.1	0.77						
	Gel 3	52.7	0.79	43.0	0.74	38.7	0.72						
	Gel 1	29.9	0.71	35.9	0.77	38.0	0.72	15.5	0.74	33.2	0.72	29.9	0.73
Rabbit 3	*Gel 2*	32.4	0.72	47.2	0.81	23.6	0.70	28.8	0.72	28.8	0.75	35.0	0.79
	Gel 3	23.3	0.73	19.7	0.69	10.0	0.6	18.3	0.71	16.5	0.71	17.6	0.70

*Mean matching percentage: **32.20% (SD=11.45%; SEM=2.73%)**

3.3 Technical Variance

The reproducibility of the gels was confirmed using analytical variances allowing comparison of the paired spots which were matched on each replicate gel. Overlay of gels from same batches of rabbits were detected and systematically matched using the commercial software (2D Platinum Image Master). The matching percentage of the replicates was found to be more than 50% (Table 2).

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Table 2: The matching percentage of spots and their correlation (r^2) between gels (G1, G2 and G3) replicates (n=3) from same batch of rabbits for the intra-sample variation study.

Rabbit 1				
	G1		*G2*	
	Matching Percentage	*Correlation*	*Matching Percentage*	*Correlation*
G2	51.60%	0.805		
G3	53.07%	0.718	51.20%	0.766
Rabbit 2				
	G1		*G2*	
	Matching Percentage	*Correlation*	*Matching Percentage*	*Correlation*
G2	66.50%	0.814		
G3	44.30%	0.708	41.40%	0.713
Rabbit 3				
	G1		*G2*	
	Matching Percentage	*Correlation*	*Matching Percentage*	*Correlation*
G2	64.30%	0.865		
G3	53.60%	0.833	50.00%	0.856

*Mean matching percentage: **52.89% (SD=8.18%; SEM=2.20%)**

3.4 Reference Proteome of Adult Rabbit Chondrocyte

Chondrocytes chemically disrupted in a 2-DE compatible lysis buffer to release cellular proteins, yielded protein at a level of 1.5 µg/ml. Gel triplicates were established from joint cartilage extracted from individual rabbits to verify the reproducibility of the protein pattern (results shown in section 3.3). A very similar proteomic profile can be identified in all protein maps replicates (Figure 7). A total of approximately 1800 to 2000 spots were detected from each gels. To further support the reproducibility of our reference map, protein spots (n=10) which appeared to be in abundance were randomly selected from the gel and excised. The same identical (position) spots were excised from the gel replicates (N=2). These spots were sent for protein identification by MALDI-ToF mass-spectrometry at the Genome Centre, University of Hong Kong. The proteins identification was carried out on the basis of peptide matching after in-gel digestion with trypsin. All spots (n=10) identified in the gel map and its respective spot duplicates in the replicate gels (N=2) were of same identity (Table 3).

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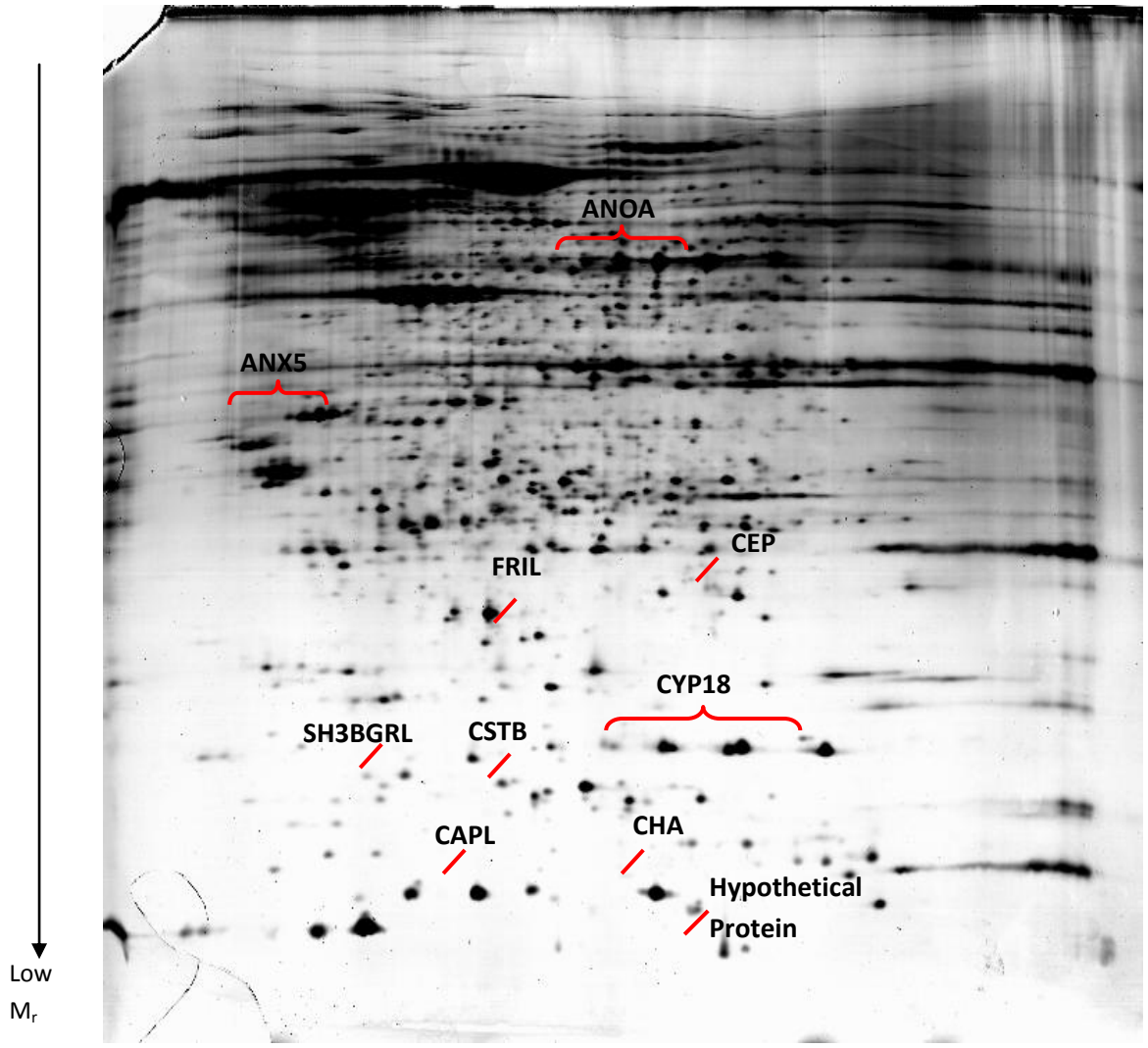

Fig 7: Two-dimensional electrophoresis gel of healthy/non-diseased chondrocytes derived from adult rabbits. Random identification of spots using mass-spectrometry and NCBInr database reveals identical proteins (n=10) at respective replicates (N=2).

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Table 3: Identification of abundance proteins expressed in chondrocyte proteome for reproducibility analysis. Spots (n=10) were randomly chosen and excised from gels (N=2) and identified by mass spectrometry and NCBInr database.

Spots ID	*Accession No.*[#]	*Description*	M_r *	*pI**
ANX5	gi | 260138	Annexin V	35992.5	4.86
ANOA	gi | 4927286	Alpha-enolase	47589.4	6.44
CEP	gi | 126723727	Cephalin (Phosphatidylethanoamine)	21094.7	6.59
FRIL	gi | 156119344	Ferritin light chain 2	20119.2	5.7
SH3BGRL	gi | 78369645	SH3 domain binding glutamic acid-rich protein	12768.3	5.22
CYP18	gi | 126722924	Cyclophilin 18	18053.8	6.96
CAPL	gi | 545707	S-100-related calcium binding protein	11895.8	5.88
CHA	gi | 34811429	Chain A, solution structure of rabbit Apo-S100a11	11404.8	6.69
CSTB	gi | 126722940	Cystatin B	11142.5	6.04
Hypothetical Protein	gi | 28189917	Similar to Polyubiquitin	19318.5	9.1

[#] Accession number according to NCBInr database

* Predicted Mr and pI according to protein sequence to NCBInr database

4. DISCUSSION

This study was specifically designed and conducted in order to establish a replicable, reproducible and reliable proteome of chondrocytes 'in vitro' along three important parameters: first by standardizing the number of batches and replicates within each batch, secondly, by statistically determining the heterogeneity of chondrocytes due to biological and technical variability and thirdly, by re-confirming the confidence of reproducibility by identifying spots in replicate gels using mass spectrometry analysis.

Although previous studies have elaborated on cartilage protein mapping from a variety of species [13,14], but the results of this study clearly establish the first protein map for adult rabbit chondrocytes derived from healthy/non-diseased cartilage. It is important to note that protein mapping of tissues from various species is just as important as that established from human tissues. In vitro cell cultures have served as highly useful and relevant models for studying mechanisms involved in regulation of responses to growth factors and cytokines as well as in understanding the pathogenesis of major joint diseases such as osteoarthritis. Representative studies include cartilage tissues extracted from pigs [21], canines [22] and goats [23]. In many animal studies, the rabbit has been successfully used as an appropriate model for cartilage disease [24,25,26]. However, with proteomics becoming a rapidly evolving and relevant technology today, proteomic analyses of rabbit cartilage have never been clearly established. The data from our study have created a benchmark reference which can be used for future studies on proteomics involving rabbits as diseased cartilage models. Our study demonstrates that variability between individuals (also known as inter-subject variability) can be big with consistency between detected proteins being as low as 10%. This is a likely consequence of biological variance related to the heterogeneity of samples and also to variability in cell growth. Nevertheless, if only abundance proteins were taken into consideration for comparison of these samples, proteome analysis was able to detect them consistently in all replicates (100%), denoting very high reliability of the described method of analysis.

Despite much interest in cartilage proteomics, direct proteomic analysis of this tissue is difficult since highly anionic proteoglycans, the major component of cartilage extracellular matrix interfere with IEF separation [13,6]. The problem was overcome by removing proteoglycan content from the samples using various methods. [6, 27, 28] In this study, proteoglycan

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was precipitated using acetone, trichloromethane and hydrochloric acid method. This technique works by quantitatively precipitating proteins while leaving behind in solution interfering substances such as proteoglycan, salts, detergents, DNA and lipid[29]. However, pre-treatment of samples for proteoglycan removal or any IEF interference molecules may result in loss of some of the proteins from the samples.

Deciphering the protein composition of complex biological extracts or determining relevant polypeptides is frequently hindered by large dynamic protein concentration ranges. The presence of high abundance proteins suppresses the signal of low-abundance proteins. In addition, the rarest proteins are frequently below sensitivity levels of available analytical methods for detection and identification. Therefore, in this study the optimal protein concentration for analysis was carefully determined by plotting the relationship of protein concentration against the number of proteins expressed in the chondrocyte proteome in order to establish a global protein map. The results revealed that by simply increasing protein loading, more spots were visualized as expressed on the gel (Figure 2). However, beyond a specific protein loading (100 µg), spots expressed on the gel remained unchanged and in fact appeared slightly reduced in spots number. Thus, differences in total protein concentration can influence expression and detection of cartilage-derived cellular proteins on the gels. Previously described as "overloading" and "underloading" of proteins, these technique-sensitive phenomena explain why proteins can sometimes visible or diminish with replicates[30]. In cases of protein overloading, merging of spots by co-migration[30] results in poor separation of proteins of fairly similar molecular weight. In contrast, "underloading" of protein results in invisible spots to the naked eye due to low protein presence to allow adequate visualization.

Volume percentage relationships of spots between gel replicates were also determined in this study. High correlation coefficients were able to be established between spot volumes of the different replicates. It was important that such correlation was independent of spot intensity but based only on the spot volume. This would otherwise have resulted in a biased outcome as high intensity spots present would be given preference based on mere appearance. Therefore, the results attained in our study would have indirectly eliminated the possibility spots resulting in 'false positives' of the detected proteins. These methods were further improved by critically (visually) examining each of the spots and manually removing those which may increase the variability as a result of an error in computational interpretations.

Two-dimensional electrophoresis has inherent limitations, particularly for the analysis of primary samples (cell or blood serum). Variability was estimated to be high in 2-DE [31] which can be linked to both the biological as well as the technical phases. It is therefore necessary that a analytical experiments are well designed so that differences between protein expression patterns due to inherent heterogeneity of samples are not masked.[32] Biological variability is expected to be high in 2-DE in terms of number of spots detected and variance in the spot volume.

Replication is necessary to assess and increase the precision of subsequent analysis results[33, 34]. In this study, it has been shown that the percentage of gel matches for technical variances are higher than for biological variance. This can be attributed to improvement in technical variability by optimizing and standardizing techniques as in this study, for example by imposing strict standards for cell preparation and protein solubilization prior to the 2-DE.

From the current study, about 1800 to 2000 spots were consistently present and detected from the chondrocyte proteome of all samples (although not all were located at the same position) denoting a large number of proteins are present in these cells. However, based on the literature surveyed, some of these proteins have yet to be described and may also include undiscovered novel and significant cartilage proteins, which are worthwhile pursuing further in future research. To increase the confidence on the reproducibility of our chondrocyte proteome, analysis of randomly selected spots were identified using mass spectrometry (MALDI-ToF). The same spots from the gel replicates were also identified. We found that, all spots identified showed 100% identical peptides within respective replicate gels, providing further evidence that certain proteins (especially those in abundance) are useful as key protein markers in chondrocytes. However, further study involving higher numbers of replicates would be necessary in order to determine the necessity of having replicates for mass-spectrometry to increase the reliability in protein identification confidence levels.

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5. CONCLUSION

This study proves that proteome analysis is reliable in determining global protein expressions consistently at an average of 52.89±8.18% for intra-sampling replicates and between 32.20±11.45% for inter-subject comparisons. However, when only high abundance proteins were accounted for each of the samples, protein expressions were consistently present (100%) in all replicates. These results provide a high degree of confidence in the proteome profiling of normal adult chondrocytes; demonstrating that the techniques carried out in this study are reliable, replicable and reproducible.

6. ACKNOWLEDGMENTS

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