MONITORING STEM CELLS IN PHASE CONTRAST IMAGING

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ABSTRACT

Understanding the mechanisms behind the proliferation of Mesenchymal Stem cells (MSCs) can offer a greater insight into the behaviour of these cells throughout their life cycles. Traditional methods of determining the rate of MSC differentiation rely on population based studies over an extended time period. However, such methods can be inadequate as they are unable to track cells as they interact; for example, in autologous cell therapies for osteoarthritis, the development of biological assays that predict in vivo functional activity and biological action are particularly challenging. Here further research is required to determine non-histochemical biomarkers which provide correlations between cell survival and predictive functional outcome. This paper proposes using a (previously developed) advanced texture-based analysis techniqueto facilitate in vitro cells tracking usingtime-lapsed microscopy. The technique was adopted to monitorstem cells in the context of unlabelled, phase contrast (PC) imaging, with the goal of examiningthe cell to cell interactions in both monoculture and co-culture systems. The results obtained are analysed using established exploratory procedures developed for time series data and compared with the typical fluorescent-based approach of cell labelling. A review of the progress and the lessons learned are also presented.

1. INTRODUCTION

In the past decade, there has been considerable expansion of the use of embryonic and adult stem cells in tissue engineering and regeneration. In arthritis research, for example, there have been several successful trials of procedures that repair damaged knee cartilage tissue using autogolous mesenchymal stem cells (MSCs) [1]. These studies have been shown to improve the lifespan of the arthritic knee, reducing the need for more invasive surgical interventions. As these techniques become more widespread, there is a growing need for automated tools to characterise the behaviours of such cells grown in vitro in high-throughput, automated systems. At present, however, there is a significant disparity between the laboratory based methods of understanding the use of stem cells and clinical best practice.

Conventionally most cell imaging based studies have used fluorescent markers to enhance the images. Whilst this offers great insight into the functions of the proteins at the molecular level, the use of the fluorescent markers alters the fundamental biology of the cell,making them unusable for subsequent cell based therapy. More relevantly, from an image analysis viewpoint, the application of time-lapse fluorescent microscopy imaging to capturing the dynamics of live cells is further inhibited by *phototoxicity* and *photobleaching*. Phototoxicity occurs in fluorescent microscopy as cells containing fluorescence are repeatedly exposed to illumination and in response the fluorescent molecules tend to generate reactive chemical species such as oxygen radicals. These free radicals damage subcellular components, and thus often affect cell fate and behaviour [3]. Photobleaching or loss of fluorescence signal due to the loss of fluorophores' ability to fluoresce during time-lapse imaging limits the number of image acquisitions, thus limiting the long-time monitoring of cells [4,9]. Consequently, the automated analysis of non-fluorescent microscopy images, most notably phase-contrast microscopy (PC) images, offers a more practical means to realise a long-term monitoring of live cell behaviour with minimal perturbation and human intervention. It is therefore critical that we are able to create tools which can monitor cellular events such as growth, migration, etc, and thus track individual cells using this imaging modality.

Computationally, analysing images with PC microscopy presents several challenges. In particular, the typically low*signal-to-noise* ratio present in such images affects the quality (and effectiveness) of the segmentation algorithm needed toseparate (bio)marker-free cells from the background of an image. Indeed, the use of entropy based algorithms has previously been developed specifically to address this problem [5], resulting in a more effective cell tracking procedure which allows the phenotypic state of the individual cell to be computed and linked to their dynamic [6]. Here, a quantitative characterisation of cell morphologiesand dynamics could help to localise dynamic cellular events, including mitosis, apoptosis etc [7,8]. As such, the need for automated tools to study dynamic cellular systems, particularly withheterogeneous cell types and populations, at single cell resolution is apparent.

The remainder of this paper is organised as follows. Section 2 describes the background/motivation for this study which summarises our earlier work relating to the development of the automated tracking algorithm as described above and, more relevantly, the use of a commercially available image analytics software platform [11] upon which analysis of our results described in latter sections of this paper are based. Additionally, the investigative approach enabling the study and characterisation of heterogeneous cell types in mono- and co-culture systems is also described. The analytical procedure to studythe tracked features of the individual cell including both spatial and temporal variables that are related to the mapping of cell phenotype trajectories is described Section 3, which also covers the experimental setup and testing conducted in this study. The results are discussed in Section 4, where comparisons based ona manually constructed ground truth are discussed, highlighting the characteristics of the co-culture systems developed. A summary which includes concluding remarks and future research directions is presented in Section 5.

2. BACKGROUND AND RELATED WORK

Automated (visual) tracking of cell population in vitro using (PC) time-lapse microscopy is vital for thequantitative and systematic study of cell behaviours based on thephenotypic traits of interest extracted during the course of a biological experiment. The spatiotemporal characterisation of (2-D) morphology and cellular processes and events which is used to analyse the individual cell in atypically heterogeneous cell system can offer important capabilities to resolve the complex dynamics of such a system, whilst avoiding the generalisation of results associated with traditional population-based assays [10]. In our current investigation of stem cell co-cultures in the context of ACI for cartilage injuries, we have adopted two automated cell tracking tools incorporating (1) our recent work [5] and (2) a fully integrated and commercial image analytics software suite available on an automated PC imaging platform (CellIQ-2®) from which all the time-lapse microscopic image sequences presented in this paper were obtained [11]. We believe that techniques and processes developed have application to other soft tissue engineering [12].[1]

2.1. CMT's Image Analyser (Version MA4.3.0.0)

The Image Analyser is an integrated software platform developed by the Chip-Man Technologies Ltd (CMT Ltd, Finland) which enables the detailed computer analysis of images of label-free live cells captured by its time-lapse microscopes for observing cell cultures. Briefly, the analyser allows users to examine the images taken previously from themicroscope and create a video that combines successive images of adjacent regions. In addition, it has the unique capability to create sample libraries to include different cell types and/or regions of cells from which a protocol can be 'trained' or refined to identify the individualcell types. This is illustrated in Figure 1 below. By building a library of,say, 100-150 cells of each type, for example, it is possible to use the inbuilt tools to track the individual cell, compute its morphological features and subsequently summarise its movements over the course of the experiment.

[1]The rationale behind using cocultures in the regeneration process of cartilage, which has been thought to only contain one cell type, may have appeared counterintuitive until bone marrow-derived MSCs were shown to have chondrogenic potential; i.e. the ability to form cartilage. Indeed, a direct coculture of MSCs with chondrocytes have been shown to increases growth of chondrocytes and chondrogenic differentiation of the MSCs [12].

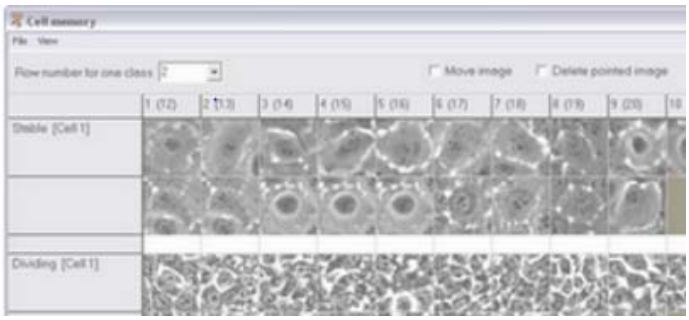

Fig. 1: An example library constructed by the Analyser .Depending on the application in hand, it should be noted that a library is often constructed for each cell type and, as such, the technology is not specific to any one type of cell,and can thus be used for a variety of studies.

In this study, the Image Analyser was used to track a total of 24 cells- 12 articular chondrocytes (ACs) and 12 MSCs, in two identically constructed co-cultures, plus 12 in each of two monocultures designed for each cell type. The process of tracking these cells and quantifyingtheir movements over time was achieved in three main steps; namely, (i) Creating a sample library, (ii) Checking the viability of the sample library on a selection of images across each time frame, and confirming visually that the individual cells are correctly segmented and labelled, and (iii) Enabling tracking to generate results for the tracked cells. Broadly speaking, a sample library was specifically constructed for each culture and consisted of a collection of exemplars of the relevant cell type(s) of interest as selected by the user. Between 100 and 150 samples were provided for each celltype/class, including where applicable, either or both of MSCs and chondrocytes to form the basis of the protocol for each cell culture. Additionally, in order to improve identification results, the exemplars also included the nucleus of each cell typewhere this was feasible.

In passing, once the first iteration of the (ii) was completed, it was found that, due to the uneven lighting of the phase contrast images, parts or regions of the background had been segmented and mislabelled as valid cells. To counter this, a third class named *Background* was also created, with 20 samples or exemplars selected from therelevant parts of the background. Using this additional library, the cell protocol was rerun until the number of correctly labelled cells was deemed adequate. Here, the cells in the library were edited to remove cells that were too similar between the groups during this iterative process until the all the tracked cells were correctly labelled. Figure 2 depicts an example for one of the libraries constructed and the corresponding results displayed to the user.

Once the cells have been correctly segmented and labelled, the analyser can perform the relatively more complex task of cell tracking. The latter enables the cells of interest to be selected from the first image of the time-lapsed sequence, with each of these cells given a label in order for it to be tracked over the specified image sequence. The tracking is the performed automatically (using a propriety shape recognition algorithm)to enable movements of the individual cells to be computed and recorded for the specified length of the image sequence as determined by the application in hand. The output is finally shown to the user, as depicted in Figure 3.

In addition to the capability of tracking cells as described above, the Analyser also generates several outputs that summarise the results of the tracking performed, most of which are based on population-based analysis. These include, for example, the *Rose* chart which plots the proportion (%) of the individual cell population against theangular displacement or directionof movement (0-360 degrees) from the start to end points over the course of tracking and, similarly, the 2-D plot which displays as a percentage of the cell population/groups, the average distance of the movement (in micrometers) at each time frame. The Analyser also generates an excel spreadsheet that records at each time frame the (x,y) position, displacement and speed of the individually labelled/tracked cell over the course of tracking.

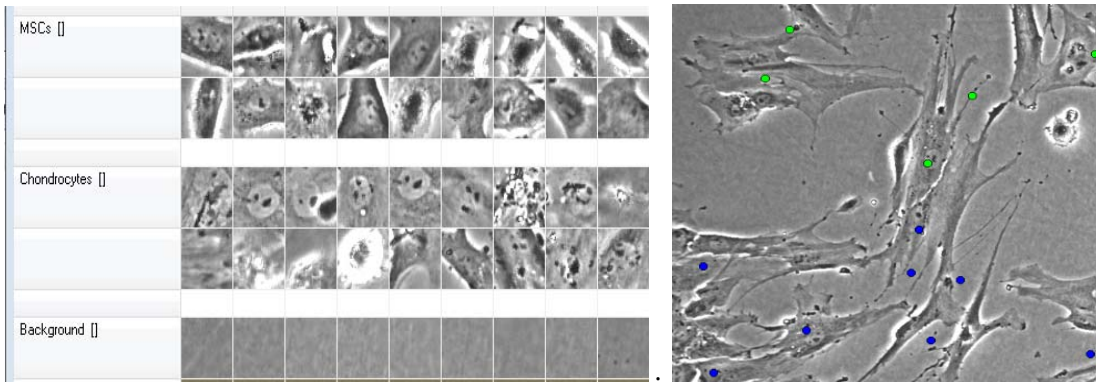

Fig. 2: (Left) The first part/section of the sample library constructed for the co-culture to define, from top to bottom, MSCs, chondrocytes and background. (Right) Labelled cells based on the constructed library are displayed in each image/time frame of the specified image sequence, with blue dots representing the MSCs and green the chondrocytes respectively.

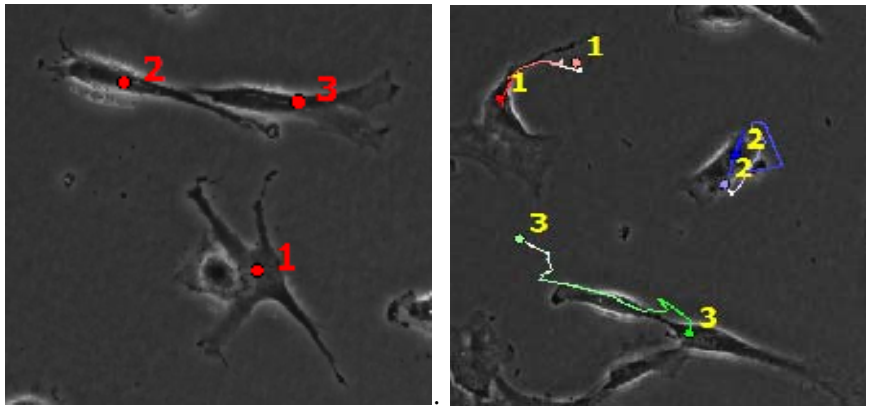

Fig. 3: (Left) An example showing the individually labelled cells at each time frame as a result of the tracking performed. (Right) Labelled cells as shown in the left figure, with the individually marked path of movements for each tracked cell covering the user specified time period of interest.

In passing, it should be noted here that,for the purpose of labelling, the full image of the individual cell is not required in general; indeed, users are often required to specify the maximum cell diameter of the individual cell as well the minimum cell distances between cells interactively during the refinement steps of (i) and (ii) as described above. Consequently, the geometric or morphological information including particularly the exact outline (or silhouette) of the individual cellis unavailablefollowing the implicit segmentation process required for (ii).

2.2. NCC with entropy segmentation cell tracking algorithm

To enablethe study of dynamic cellular systems at single cell resolution, a dedicated cell labelling and tracking tool was developed recently [5]. Specifically, a more sophisticated cell/label linking procedure - namely, the *Normalised Cross Correlation* (NCC) procedure, which facilitates association of the individually labelled cells over consecutive time frames has been incorporated with the entropy-based segmentation algorithm previously developed[2]. The procedure describedsought to address much of the shortcoming of template matching based proceduresand has previously been

[2] CMT's Image Analyser uses template matching for segmentation and the *nearest neighbour* algorithm to link cells in consecutive frames of the tracked image sequence.

used in a number of image tracking applications. As such, it was shown to be able togenerate the best position of the target imagefor the search image (in the preceding frame), from which a cell that has a visual appearance most similar to the target imagecould be located. An exampleused for this study is shown in Figure 4 (left) below.

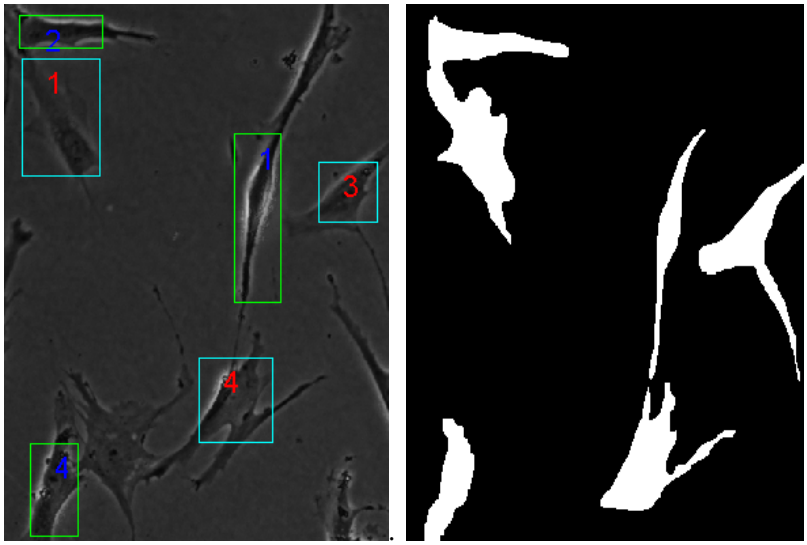

Fig. 4: (Left) An example showing the individually labelled cells using the NCC based procedure for cell linking and tracking. (Right) The entropy based segmentation was used to separate background from the labelled cells as shown on the left.

In addition to its demonstrable effectiveness, the tool intrinsically segments the individual cells before they are labelled and linked over successive frames of the time-lapse microscopy image sequence, thus allowing quantitative measures for tracking and motion analysis of each cell to be performed; i.e. studying and analysing the specified image sequence of interest at single cell resolution. More significantly, as the tracking algorithms captures the entire cell shape at each time point (from which a position estimate is derived, commonly by computing the centroid), measurements ofthe individually segmented cell object as a function of time also allows for the computation of a host of measurescharacterising the cell morphology; see Figure 4 (right) above. Here, a distinction can be made between measures of size and orientationversus measures of geometric complexity; see earlier work on granulometric studies [5]. Examples of the former include the perimeter, area(volume), the major/minor axes etc. Measures of geometriccomplexity include circularity, eccentricity (or ellipticity) and convexity or concavity. More sophisticated analysis of morphologycould also be achieved, for example, by decomposing the shape based on Fourier and Zenike decompositions, principal component or independent component analyses; see [13] for detailed description.

By tracking cells individually (as well as collectively in a population), spatiotemporal cellular events such as mitosis, differentiation and apoptosis can be accurately localised and recorded alongside multi-parameter quantitative data obtained from the available (three/four) dimensions to help study complex and dynamic cellular processes. Further, spatiotemporal kinematics with motion attributable to proliferation (migration and/or growth) can be defined More importantly, quantitative image/data-driven methods to reveal statistically significant differences in cell behaviour in response to stimuli, including cell to cell or cell to ECM properties that can be measured with cell displacement, speed, persistence, etc. An example of such measurements as described above is given in Figure 5.

3. EXPERIMENTAL STUDY AND TESTING

Over the past four decades, significant advances in tissue engineering have alreadybeen made by employing co-cultures of stem cells, particularly adult stem cell such as the MSCs, together with terminally differentiated cells or target cells.

Methods to control and employ stem cells have been of paramount interest [14,15]; in orthopaedic soft tissue engineering and regenerative medicine, for example, a direct co-culture of MSCs with ACs has been demonstrated to provide increased growth of ACs and chondrogenic differentiation of the MSCs [16]. Here, co-culture systems control the behaviour and actions of cells through the interaction of the multiple cell types commonly referred to as the target cells (ACs) and assisting cells (MSCs). Generally speaking, target cells are those that will eventually compose the engineered tissue and are responsible for the tissue's function, whilst assisting cells guide the target cells to display a range of desired behaviours, including proliferation or differentiation, matrix production or organisation by direct cell to cell contact, adhesion of cells to extracellular matrices (produced by the assisting cells) and/or secretion of signalling molecules. However, the underlying interactions between assisting and target cells are often too complex to be elucidated, still less to implement through exogenous control.

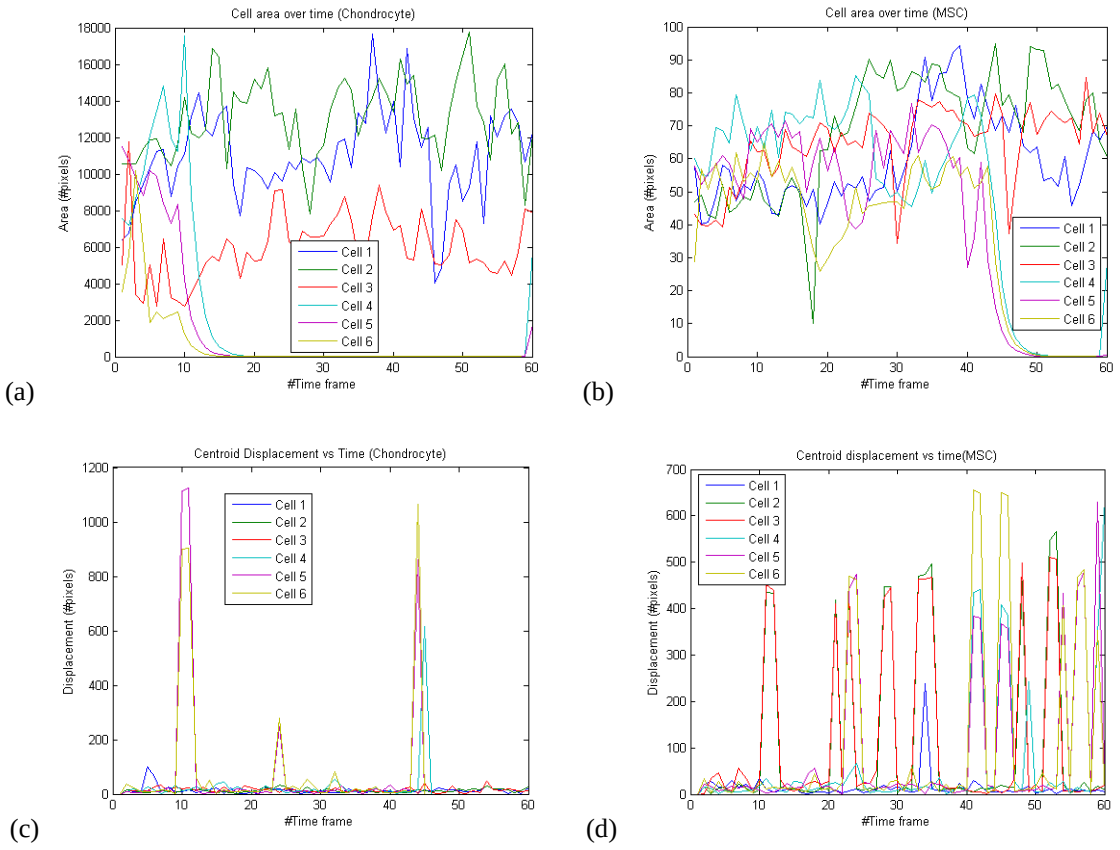

Fig. 5: An example of the spatiotemporal measurements for the (two) different cell types (chondrocyte and MSC) in the co-culture studied in this work. From left to right and top to bottom: (a) Cell areas measured in number of pixels (#pixels) over time for chondrocytes. (b) As (a) for MSCs. (c) Centroid displacement (# pixels) over time for chondrocytes. (d) As (c) for MSCs.

The experiments developed in the study sought to examine the current view that coculture strategies increasingly outperform their monoculture counterparts with regard to chondrogenesis, and to attain chondrocyte phenotype stability in vitro; for example, see [2] and [17-19]. By studyingat single cell resolution (via tracking) the phenotypic state of the ACs and MSCs in monocultures and/or co-cultures, our work principally concerns the computation of biologically meaningful quantitative measures such as motility and morphology, as biomarkers, that would facilitate phenotypic characterisation (or profiling) of these cells.

3.1. Experimental setup

To help explore the relevant biometrics of the ACs and MSCs as described above, two monocultures consisting of articular chondrocytes and MSCs respectively, plus a co-culture where the two cell types are directly mixed have been constructed. Due to their simplicity and ease of use/control, this was achieved in two-dimensional (2D)systems with a culture environment where the respective single and mixed-population monolayers were used to study the specific aspects of cellular interactions and behaviours of interest. Additionally, fluorescent labelling was also used in order to establish a ground truth[3]. In all cases, the cells were grown to their seventh passage in the laboratory, with 50,000 cells seeded in each well; i.e., in the case of the ACs/MSCs co-culture, 25,000 of each cell type were present.

As in our earlier work on cell tracking, the microscopic image/data sequences studied here were obtained using the high-throughput time lapsed microscope[4] equipped with a high precision motorised *z*-stage that enables the automated acquisition of the so-called*all-in-focus* images frame-to-frame over time. Coupled with the use of PC optics, the imaging platform allows the integration of high speed and continuous focusing software to keep cells in focus before their acquisition. These images were then segmented with individually tracked cells linked as described in section 2.2 using MATLAB® analysis code developed in-house. In essence, the tracking implemented connects cells from one frame to the next in the image sequence captured over the entire course of the experiment (~ 100 hours). To facilitate analysis of the individually tracked cell in each culture/well, thepositions of the individual cells are computed (and labelled) in each time frame on the basis of their dynamic (2-D)*centroid*coordinates; see figure 4. While this is an essential step for data reduction, further insights into the dynamics of each cell type were obtained by computing and studying biologically meaningful quantitative measures, as *biometric features* from these coordinates.In this exploratory study, these measures included *cell areas*, *equivalent diameter*, *movement* - scalar *displacement*and angular *direction*,*orientation*, *circularity* and *aspect ratio* (major-minor axis ratio) as determined by the *minimum enclosing box*. See [20] for details.

As with the precursor work on the Image Analyser as described in section 2, a total of 24 cells consisting of 12 ACs and 12 MSCs were tracked in the two identically constructed co-cultures constructed for this study. In addition, two further sets of 6ACs and 6 MSCs were also followed respectively in each of the two monocultures dedicated for each cell type. Finally, an individual time sequence was then collated for each of the dynamic measurements described above from the original microscopic image sequencesof these cells.

3.2 Single cell analysis

The collated time sequences for each cell as described above collectively provide the complete profile of dynamic measurements for the respective biometric features at successive (and uniformly spaced) time points/frames over the course of the experiment. As such, they have formed the basis of the exploratory analysis carried out in this study described below.

3.2.1 Preliminary exploratory analysis

Initially, the collated sequences were examined visually using simple data plots as shown in Figure 5 above. Additionally, simple descriptive statistics including mean, standard deviation, correlations, minimum, maximum, etc, were also computed with the goal to uncover consistent patterns (such as cycles/periods) and, importantly, significant trends as well as any outliers that might require further investigation. In particular, this study sought to investigate the widely debated topic of chondrogensis properties of MSCs in co-culture systems that could be attributed to (or even quantified by) the different behaviours exhibited in mono-cultures. This is illustrated by the comparative example shown

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[3] The co-coculture was labelled fluorescently and put in Dulbecco's modified Eagle's medium (DEM) with 10% FBS (foetal bovine serum).

[4]Cell-IQ2®, CM Technologies Ltd.

below in Figure 6, where the same set of measurements were obtained for the two cell types (ACs and MSCs) in their respective monocultures.

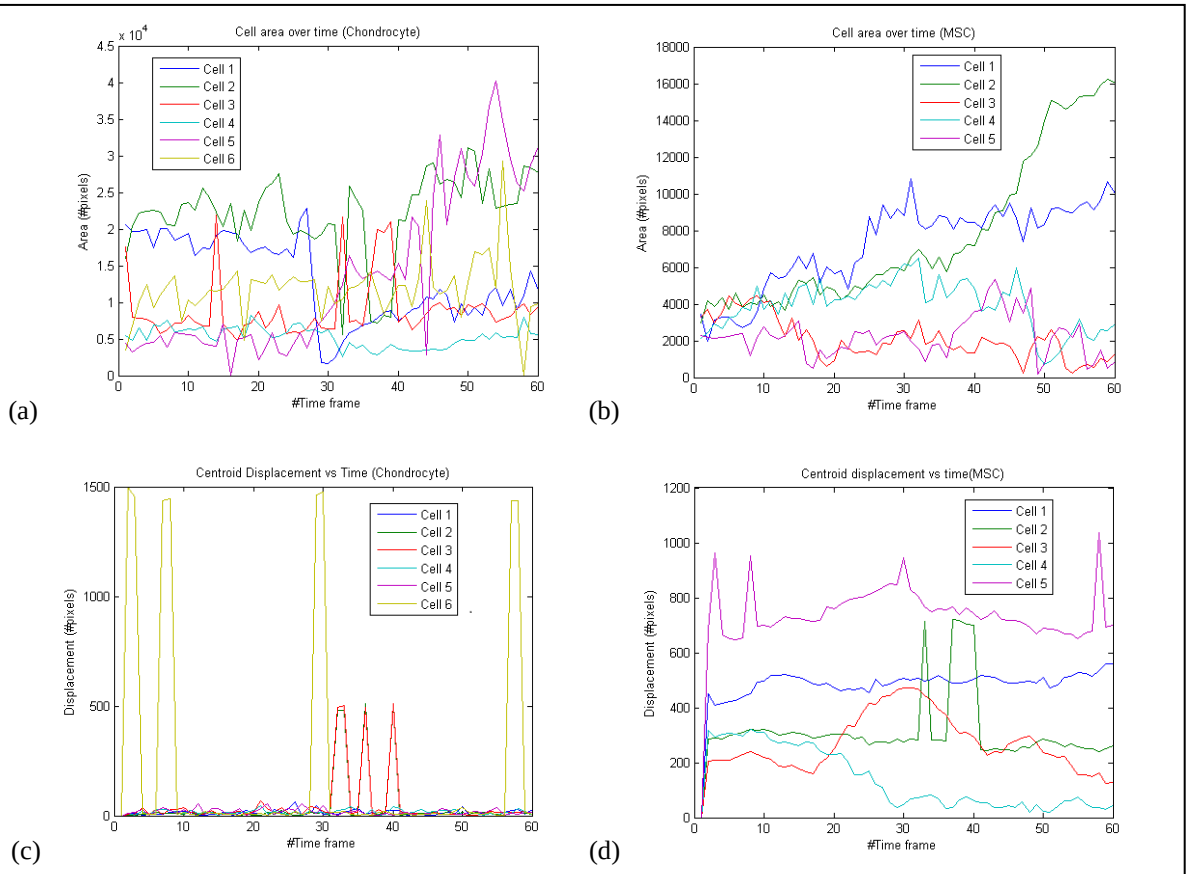


Fig. 6: An comparative example for the notably different phenotypic behaviour observed in the monocultures dedicated for ACs and MSCs; *c.f.* Figure 5. From left to right and top to bottom: (a) Cell areas measured in number of pixels (#pixels) over time for chondrocytes. (b) As (a) for MSCs. (c) Centroid displacement (#pixels) over time for the ACs. (d) As (c) for MSCs.

A cursory study of the displacement profiles of the two cell types in the mono and co-cultures (in Figures 5 and 6) revealed thatthe ACs have a lower mean displacement (#pixels) than the MSCs' over the same time period in both culture systems. In addition, the variation in displacements over the same time period (or speeds) of the individual MSCs in the co-culture appeared to be significantly larger in the co-culture.This latter observation is borne out by consistently higher variations in their speeds in the co-culture system, which is obtained by computing the standard deviations (STDs) of the individual MSC displacement profiles.

A further study of the two figures incorporating the area profiles obtained in the two culture systems appeared to show that adifferent pattern was displayed by the two cell types. In particular, whilst the ACs are generally larger in size than the MSCs, their (size) changes were also consistently larger in the mono-culture than in the co-culture system, as is evident by the calculation of the STDs of the areas for the individual size profiles measured in the two culture systems. However, the notable trends displayed by the sizes of the MSC as evident in Figure 6b were not obvious by the descriptive statistics of the STDs. The need for sophisticated analytical procedures to reveal such trends is apparent.

3.2.2 Singular spectrum analysis

Singular spectrum analysis (SSA) isnon-parametric technique which has been widely used to handle a verybroad class of time series that can contain combinations of complex periodicities,polynomial or exponential trends [21]. It has found applications for a wide range of tasks including relevantly, trend or quasi-periodic componentdetection and extraction, de-noising, forecasting and change-point detection.The basic SSA method consists of two complementary stages: *decomposition* and *reconstruction*; each includes two further separate steps. A key concept in studying the properties of SSA is `separability', whose absence is often observed in series withcomplex structure. For such series and series with special structure, several variants of SSA have been developed, with single and double centering, Toeplitz SSA, and sequential SSA [21,22]. The approach used in this study was adapted from the work by [23] and consists offour key steps: (1) *Embedding*, (2*) Singular Value Decomposition* (SVD) of the so-called *trajectory* matrix, (3) *grouping* and (4) reconstruction. Briefly speaking, a *Hankel* matrix is constructed from the time series of interest in step 1,by sliding a window (of width L) that isshorter in length than the original series. This matrix is thendecomposed into a number of elementary (rank one) matrices ofdecreasing norm by means of SVD (step 2). The approximation of the matrix is obtained by selecting and grouping these elementary matrices necessary for the extraction of the behaviours or tendencies of interest from the original time series (i.e. step 3). The latter is reconstructedin the final step by retaining the desired number of elementary time series (r) commonly referred to as the *principal components* via the diagonal averaging of the associated elementary matrices. An example which applies this algorithm to the time series depicted in Figure 6b is given in Figure 7.

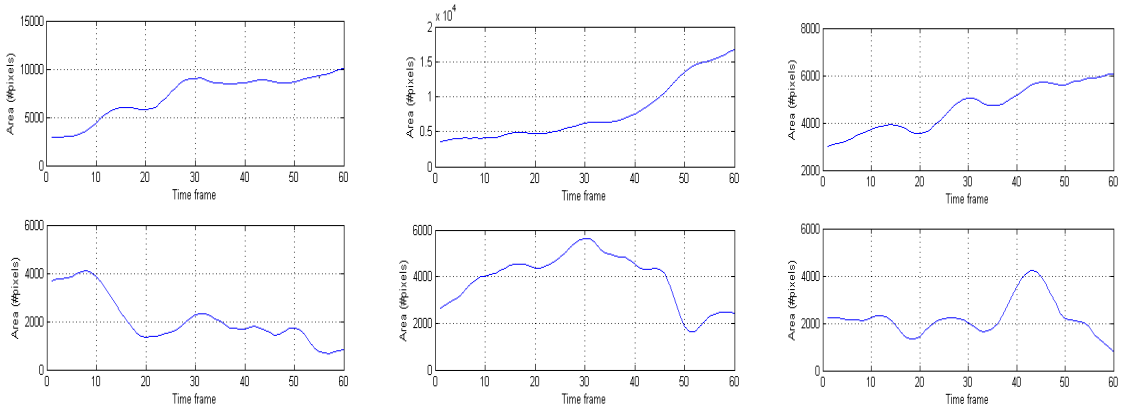


Fig. 7: An example of using SSA (L= 5, r=1:2) to extract trends in the area measurements for the individual MSCs shown in Figure 6b. The cell labels are reordered to illustrate the apparently upward and downward trends which are shown in the top and bottom row of the figure respectively.

As described in Section 3.1, a total of 36 cells with both ACs and MSCs were selected for this study from two culture systems, including the two monocultures developed for each cell type and two identically constructed (direct) co-cultures where the ACs and MSCs were mixed. For each cell, dynamic biometric features including seven measurements (cell area, displacement, etc) were computed over the course of this study and the respective time series were constructed for further exploratory analysis. The descriptive statistics as described in Section 3.2 is summarised in Table 1. below.

A few notes are in order. First, the ACs are much larger in size when compared to MSCs in both cultures; see Area and Perimeter measurements. Second, the averaged displacement/movement of the MSCs appeared to be significantly larger in the monoculture than in the co-cultures, where such measurements were much smaller and perhaps somewhat surprisingly, in line with the measurements obtained for the ACs (see marked averages in green and cyan). Third, the variations in shape and sizes of the individual MSCs as measured by the standard deviations of the respective measurements across the individual cells (under STD (cell)) in both culture systems are relatively and consistently larger the ACs', which confirms the general belief that MSCs are mixtures of different cell types. Last and perhaps more

interestingly, asides from the overall larger size, the shape of the ACs appeared to look more like the MSCs when they are mixed in the co-cultures; see marked averages (in yellow).

TABLE 1

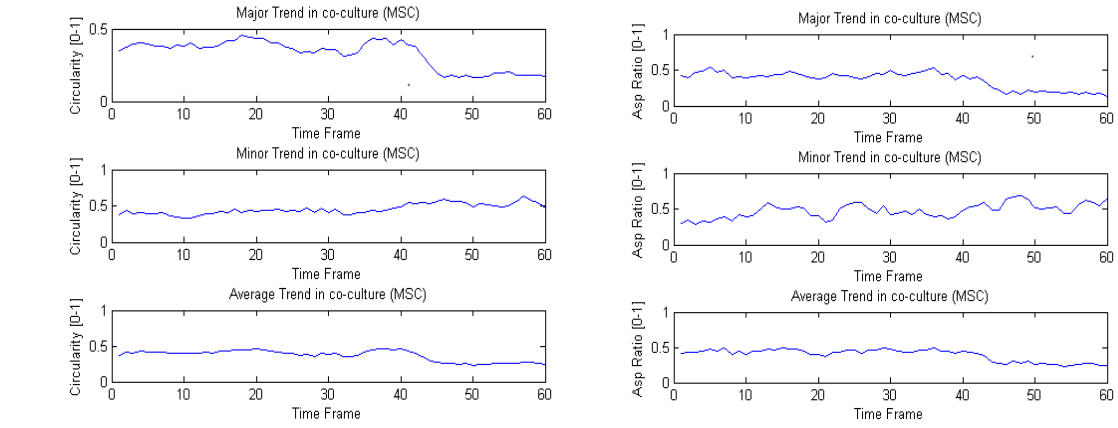
Dynamic Measurements	Monoculture						Coculture					
	Articular Chondrocyte (AC)			MSC			Articular Chondrocyte (AC)			MSC		
	Mean	STD	STD (cell)	Mean	STD	STD (cell)	Mean	STD	STD (cell)	Mean	STD	STD (cell)
Area (pixel)	12454	5272	5637	4640	2005	2703	9969	4030	3129	3280	1907	1487
Displ (pixel)	57	129	73	387	108	231	47	128	33	51	115	39
Displ (radian)	3.094	1.730	0.169	3.076	1.808	0.136	2.862	1.893	0.340	2.920	1.830	0.456
Orientation (radian)	0.156	0.646	0.475	-0.33	0.942	0.255	0.060	0.860	0.602	0.167	0.641	0.219
Circularity	0.277	0.121	0.086	0.460	0.153	0.114	0.570	0.151	0.068	0.366	0.196	0.115
Perimeter (pixels)	797	262	280	358	121	102	475	149	85	308	160	113
Aspect ratio	0.448	0.200	0.0811	0.486	0.176	0.132	0.568	0.172	0.061	0.392	0.235	0.124

A closer examination of the related shape profiles of the MSCs measured in both culture systems confirms the last note above and, importantly, offers a greater insight into the dynamic process involved. Using the SSA technique described in the preceding section, it was shown both the circularity and aspect ratio measurements have revealed some demonstrably consistent trends in the change in shape of the MSC in the co-culture. This is summarised in Figure 8a where the decline in the circularity measures over time, and the related but different measurements for the aspect ratio computed for the individual cells, is apparent. Indeed, the same analysis applied to the change in orientation over successive time frames further supports this finding and, collectively, they have revealed dynamic patterns which were absent in ACs in either of the two culture systems. See Figure 8b for description.

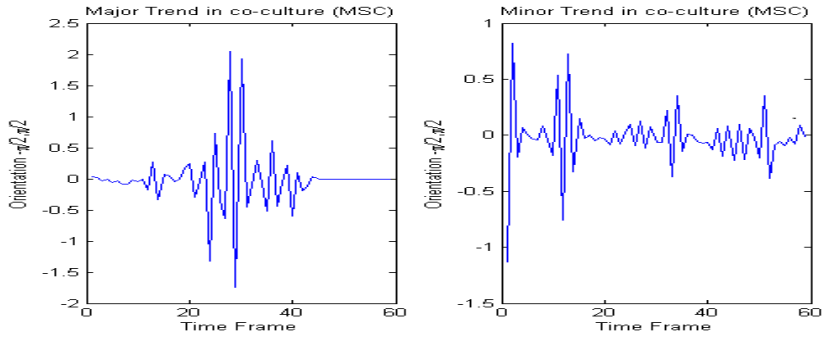
5. CONCLUSIONS AND FUTURE WORK

Quantitative measurements that describe cell motility and morphology, as biomarkers, were examined with the goal to assist the phenotypic characterisation or profiling of ACs and MSCs, particularly in the direct co-culture system where the two cell types are mixed (for advantageous mutual benefits). In addition to using standard though relatively simple descriptive statistics, further studies based on the non-parametric technique of the SAA algorithm has shown to be adequate in revealing the apparent morphological changes of the MSCs that were absent in the similarly constructed monoculture. In agreement with the recent work on co-cultured ACs and MSCs (e.g. [2]), the results support the widely held view that MSCs enhance AC proliferation, while concurrently ACs promote the differentiation of MSCs. To facilitate investigation of chondrogenic markers that would enable full AC phenotype characterisation, additional work which builds on this study is needed on investigating analytical tools to extract and assess computationally tractable and

biologically meaning metrics that are obtained via automated high volume screening for functional outcomes of cell transplantation. This would necessitate a top-down approach to analyse biometric features resulted from the related studies or experiments on cellular interactions and behaviours of interest, with further refinements on the SSA algorithm described here to analyse the individually tracked cells and, possibly, improvements on the developed tracking tools.



(a)



(b)

Fig. 8: (a) (Left) The circularity trend extracted from 75% of the MSCs using SSA (L=5, r=1:2,5) (Right) The corresponding trend in the aspect ratio measurements for the individual groups (majority and minority). (b) The associated changes in orientation as measured over successive time frame, suggesting that the majority of the MSCs have undergone relatively significant changes in shape/orientation (~2x in amplitude) and over a relatively short time period when compared to the minority group.

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