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DEVELOPING STEM CELL CLINICAL TRIALS IN SPINAL CORD INJURY THROUGH ARTIFICIAL INTELLIGENCE TECHNIQUES

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Abstract: Primary characteristics that facilitate the treatment of SCI with MSCs include their proliferative potential, immunomodulation capacity, and differentiation ability. Nevertheless, traditional approaches employed to determine stem cell viability and readiness for therapeutic use are vague, detrimental, or prolonged. In this study, we present a new image analysis method for predicting viability and therapeutic potential of mesenchymal stem cells in spinal cord injury models through their detection and classification. The algorithm was developed based on phase contrast microscopy images acquired during the primary and early logarithm stages of MCS expansion concerning the specific treatment protocols designed for spine cord injury. Stem cells were identified through edge detection, thresholding, and morphological operations. In order to resolve inter-cells within clusters, H-minima transform, and Hidden Markov Models (HMM) were applied. Marker-controlled watershed techniques were used to segment out the clustered cells in order to obtain single-cell data. Consequently, morphometric and textural features were extracted, and machine learning techniques were employed in order to classify on the basis of morphological phenotypes of MSCs. The algorithm was tested externally over 899 MSCs selected from 596 culture images of spinal cord injuries. The model has proven to be 97% sensitive and 88% specific, producing 98% and 98% precision for detection and segmentation of MSCs, respectively per image. For classifying MSC phenotypes expanding during early and mid-lag phases, the AUC values were 0.99 (CI95 = 0.976–0.988) as obtained in the charts. The presented approach attributes a high level of accuracy and reliability in segmenting and classifying MSCs according to their shapes. This approach is useful for non-invasively determining the quality of MSC culture and can help in the maintenance of quality control based on the morphology of the cells in relation to the development of treatment regimens using mesenchymal stem cells for repair of damaged spinal cord tissues.

Keywords Image Segmentation, Cell Phenotype Classification, Machine Learning; Stem Cell, Monolayer Cell Culture, Viability Assessment

I. INTRODUCTION

A stem cell is an undifferentiated cell found in all multicellular organisms with the ability to self-renew and to differentiate into multiple types. Regenerative medicine and tissue engineering have been linked to stem cells in an effort to enhance patients' quality of life and overall health, particularly for those suffering from life-threatening illnesses. Three types of stem cells are known: (1) induced pluripotent stem cells (iPSC); (2) adult stem cells (ASC); and (3) embryonic stem cells (ESC) produced from early-stage embryos. These cells' capacity for regeneration stems from their ability to travel to the damaged area of the body, divide, and create daughter cells that, given the right circumstances, can differentiate into multiple cell lineages to heal the damaged tissue [1-3]. The culture environment and secretomes secreted can affect stem cells' ability to promote regeneration [4-5]. Numerous clinical diseases have been examined and even treated with stem cell therapy. However, there are risks that require additional assessment (before clinical application). These concerns include immunological rejection, misdirected or misdifferentiated cells, and the main concern, genomic instability or tumor formation [6], [7]. Although the use of stem cells in medicine is growing, the body doesn't have many of them overall. According to standard cell therapy protocols, each treatment needs hundreds of millions of MSC, which means that the cells

must expand in vitro for roughly ten weeks prior to implantation [8-12]. This means that extended stem cell growth or modification may lead to cellular senescence or even carcinogenesis in vitro, making the cells unsuitable for therapeutic application. The debilitating disorder known as spinal cord injury (SCI) frequently leaves victims with lifelong neurological abnormalities that severely lower their quality of life as shown in Figure 1. With few alternatives for real regeneration and repair, traditional treatments have mostly been supportive. Since Hippocrates, paraplegia resulting from spinal cord injury (SCI) has alarmed medical professionals and changed the lives of those affected. The primary goals of treatment have been to preserve any intact neural tissue and to lessen the impact of subsequent injuries, such as oedema, bleeding, necrosis, and demyelination. [13-18].

Microglia, fibroblasts, and reactive astrocytes in the spinal cord cause gliosis and scar formation, which restricts neuronal regeneration [19-22] Over the past three decades, stem cell-based therapies have been created and show promise for treating spinal cord injury. Since bone marrow-derived hematopoietic cells from Donald Orlic's lab at the NIH were able to regenerate an infarcted myocardium, stem cells have been extensively used for various organ systems. The three consistent effects of the many lines of stem cells are despite their disparity in biology. They are able to substitute for the dead damaged cells through their ability of multi-differentiation, which is their most important asset. Secondly, they are also capable of secreting and synthesizing anti-inflammatory factors to modulate the inflammatory response in the injured microenvironment. Finally, they secrete a variety of cytokines, growth factors, and cell adhesion factors to promote tissue regeneration [23-27]. These abundant potentials were in trial settings and gave rise to the hypothesis that the neuroregenerative effects of undifferentiated cells might be capable of addressing treatments that would yield promising results in SCI patients. In any case, the uplifting results from labs have so far not converted into genuine outcomes in these patients. Not with standing, late headways in man-made brainpower (man-made intelligence) and foundational cell treatment offer promising roads for additional compelling mediations as mentioned in Figure 1 [27].

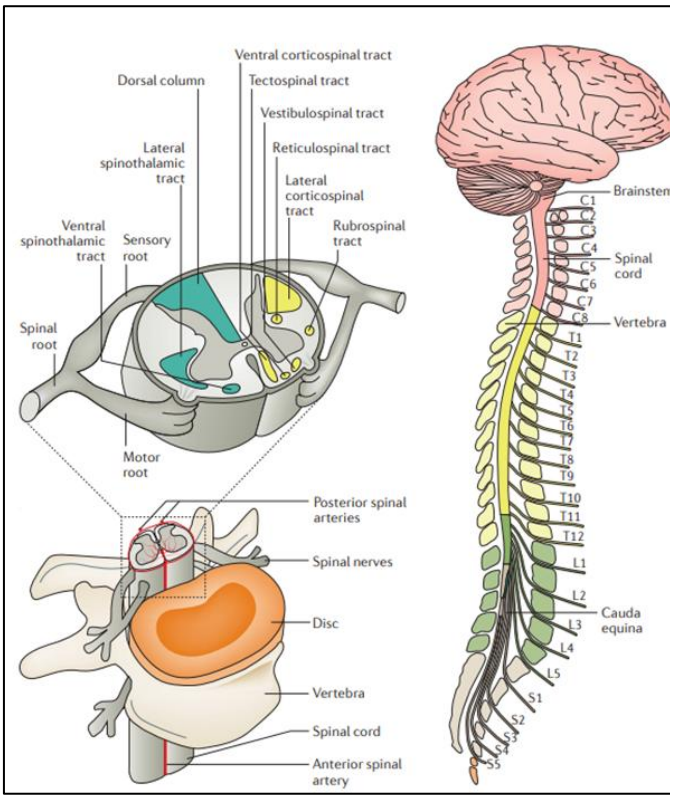


Fig.1. Spinal cord injury neurological abnormalities [1]

In conclusion, stem cells are opened with further hope for regenerative medicine and tissue engineering, especially for pathologies limited in treatment options, such as spinal cord injury. Many of these offer possibilities for cellular therapies since they could migrate through damaged areas to contribute to tissue repair via cytokine and growth factor production, among many others. However, the huge challenge of translating stem cell therapy into clinical routine practice is shadowed by immunogenicity, genomic instability, and developing tumor formation during prolonged in vitro expansion. While the advancing technology in artificial intelligence and stem cell research is hearing major potentials to up the efficacy of available therapies, actual implementations seldom yield the kinds of successes that are seen in preclinical conditions. Continued research and the clinical investigations being conducted are very important to ensure that the full therapeutic potential envisioned in stem cells is harnessed to provide hope for SCI patients and patients needing advanced regenerative therapeutic advances.

II. LITERATURE REVIEW

The application of stem cell therapy for spinal cord injury has gained considerable interest due to its potential to regenerate damaged neural tissues, induce recovery of function, and enhance the quality of life of patients. Nevertheless, transforming this profound laboratory science into effective clinical practice remains a challenge. Issues, such as complex patient heterogeneity, diverse injury characters, and the multifaceted nature of stem cell behavior in vivo, have often remained hurdles for the initiation of clinical trials for SCI therapies. AI has risen as an opinion reinforcer to overcome the aforementioned complexities since it can provide knowledge on patient stratification based on data, optimize trial design, and predict modeling of treatment outcomes. The integration of AI into stem cell clinical trials for spinal cord injury could speed up the development and validation of patient-centered, effective treatments, closing the void between laboratory success and clinical efficacy. This literature review briefly describes possibilities of AI for accelerating stem cell-based clinical trials in SCI, with chosen aspects like the way machine learning algorithms, predictive analytics, and other AI methods can best serve to improve trial design, patient selection, and therapeutic outcome success.

Stem cell therapy shows great potential in the treatment of spinal cord injury (SCI) in that it facilitates tissue repair. Neural stem cells (NSCs), neural precursor cells (NPCs) are able to differentiate into neurons, oligodendrocytes and astrocytes and thus promote myelin regeneration, neuroregeneration and functional recovery in studies conducted on animals and humans. However, the results of clinical studies have been contradictory with varying degrees of success depending on the type of stem cell and when they are used, and the degree of injury [28-30]. There are also problems associated with regeneration in SCIs such as the presence of factors that limit growth such as a low concentration of cAMP within the cells, inflammation, and the absence of neurotrophic factors. However, there are cell-based therapies aimed that do all or any of these that can establish growth facilitative conditions allowing for some recovery particularly in terms of remyelination and axon regrowth. Changes in connectivity among neurons have been seen in patients with incomplete spinal cord injuries, suggesting the possibility for some degree of recovery. For Complete spinal cord injury, on the other hand, there is not yet enough convincing proof for recovery of function [31].

The neurotrophic factors over which stem cells have control and release during the acute or subacute phases of SCI are also protective in nature by limiting inflammation and the damage to the blood-spinal cord barrier. The former is crucial to recovery for without it that will hardly be achieved, but the extent to which myelination will impact on recovery from the injury is still a question of concern. More studies are necessary in order to elucidate the relay mechanism, as well as the formation of new pathways connecting the brain and the spinal cord, which are essential for the return of function. Stem cell-based therapies for several conditions have been attempted as evidenced by active clinical trials, but there is still need for additional works to perfect such therapies [32].

New connections are being made between the brain and spinal cord as part of the recovery process from a spinal cord injury. These are made possible by the relay mechanism and ongoing clinical trials using stem cells for spinal cord injury. It has been demonstrated that the transplanted cells labeled with β-III tubulin and hNu antibodies cause neurites and the presynaptic areas of host neurons in the spinal cord to position juxta. The research indicates alludes to vital cellular mechanisms through which recovery is initiated or promoted after spinal cord injury (SCI). These mechanisms involve stem cell transplantation as well as how these transplants integrate into the spinal cord. Transplanted cells that were β-III tubulin labeled project out either as differentiating into neurons or contributing to neuronal processes. The HNu label distinguishes the transplanted human cells from those of the host. Together, these markers signify that the transplanted cells are acting as neurons in the injured spinal cord. The transplanted cells reach out their neurites (which are projection-like axons or dendrites from neurons) further in direct proximity to the presynaptic regions of the host neurons, where neurotransmitters are released to relay signals to other neurons. Close proximity is requisite for the development of new synapses between the transplanted and host neurons. After an SCI, broken neural circuits interrupt communication from the brain to the body, and any hope of recovery depends on reconnecting circuits. The passage provides the approach by which the transplanted cells can integrate into the host's spinal cord through new connections, making spatial connectivity and communication between neurons much more feasible. Such circuits might help in restoring function in the areas of the body affected by SCI by establishing neural relay mechanisms and possibly connecting the brain to the spinal cord once more [33]. For a successful functional recovery, the regeneration of this relay mechanism is just as crucial as the regeneration of the neurons. There is no way to estimate how long it will take in human beings because it has only been seen in laboratory research, where it is a very slow process. Further research is necessary to fully understand the relay mechanism and the construction of new circuitry, which are crucial for the development of connections between old and new neurons, based on evidence from animal models. In the past ten years, a number of clinical trials with stem cells obtained from various sources have been carried out on patients with spinal cord injuries [34-38].

The majority of researchers used umbilical cord or bone marrow-derived MSCs as a source of stem cells for transplantation. Others have mentioned employing human umbilical cord blood cells, neural stem cells, OECs, or Schwann cells. Although all of this research contributed significantly to our knowledge and comprehension of stem cell therapy, there were a number of issues that limited how broadly the findings could be applied and how widely accepted the findings could be. The most recent phase 2 trial, conducted by Oh et al., (2016) involved in injecting autologous MSCs intravenously into SCI patients [39-41]. During six months following the injection,

individuals showed varying degrees of functional recovery. They were able to demonstrate the safety and viability of their intervention, but they were unresearched [rove a causal relationship because the trial lacked serial imaging, histological testing, and a control group. In a review, Damianakis et al. (2022) examined eighteen papers about the transplantation of stem cells in human beings. They concluded that there was a lack of inter-study uniformity in the stem cell source, mechanism of administration, time of transplantation, and cell dose for all of these trials. The features and classifications of SCI patients as well as the follow-up patients varied between this research [42].

In mouse models of spinal cord injury, clinical trials have demonstrated the beneficial effects of combining stem cell therapy with intense physical rehabilitation. Additionally, cell transplantation of the lumbar enlargement has been proven to promote spinal conductivity as well as activity of the central pattern generator. FES provided by wearable devices has been proven to improve the functional status of SCI patients. It aims at reorganizing brain circuits and has been termed as an altered rehabilitation technique. It has been put forward that HAL suits and devices allow patients with partial SCI to magnify their limited stimulations, transform them into enormous stimuli through an exoskeleton, and offer mechanical support for joint mobility [42]. Epidural spinal stimulation (ESS) of the lumbosacral spinal cord segments has drawn a lot of attention in the last 10 years, as several studies have reported that individuals with paraplegia have been able to regain motor function with supported walking. The least obvious advantages of these trials have been the greatest: enhanced blood pressure, improved control over the bladder and bowel, improved sexual function, and improved management of body temperature. The question of whether EES and stem cell therapy could work in concert is still open, but a recent study from Lausanne suggests that they might by finding a subset of spinal interneurons that are activated by spinal injuries and support the return of ambulation when given EES. This implies that the vital populations of recovery-organizing neurons activated in spinal cord injuries may one day be enhanced by stem cell therapy [43].

Since artificial intelligence provides strong tools for data analysis and predictive modeling, it is transforming the field of SCI research and care. Personalized treatment regimens are made possible by AI algorithms' ability to evaluate enormous volumes of clinical data to find trends and forecast patient outcomes. AI has the ability to forecast consequences like pressure sores and urinary tract infection (UTIs), enabling early intervention and better patient care. Furthermore, stem cell therapy techniques are being optimized with the application of AI. The best combinations of stem cells, biomaterials, and pharmaceuticals to improve the integration and survival of transplanted cells can be found with the aid of machine learning models. The aim of this combinatorial strategy is to improve the microenvironment in which stem cell therapy is carried out, therefore increasing its efficacy. There are several clinical trials under progress to assess the effectiveness and safety of stem cell treatments for spinal cord injuries. These studies are investigating a range of approaches, such as combinatorial therapies using pharmacological agents, biomaterial scaffolds, and genetically engineered stem cells. The early findings are positive, since some research indicates that people with SCI have better quality of life and improved motor function. But there are still a lot of obstacles to overcome. It is imperative to address the key challenges of transplanted cells' long-term survival and integration. Furthermore, the ethical and regulatory environment surrounding stem cell therapy is complicated, necessitating close monitoring to guarantee patient safety [44].

Forecasting future events based on previously unseen data is crucial to the use of predictive models, especially machine and deep learning models. Its importance in regenerative medicine is similar to its objective healing system; quite simply, it is tailored medicine and has better therapeutic approaches. Predictive modeling is used in healthcare to estimate disease evolution, assess the risk of certain diseases in the population, and adjust individual treatment strategies, among other applications. Nonetheless, the inherent complexity of health care data, coupled with its volume, renders predictive modeling a cumbersome process. These models are further enhanced by AI which examines biological and clinical elements for their latent relationships as well as pattern recognition which helps envision the disease outcomes more effectively. Machine learning techniques facilitate the understanding of the elements responsible for the appearance and development of diseases hence accurate models can be devised for risk group assessment and treatment enhancement [45].

AI also creates personalized analyses and patient-specific predictive models based on specifics like proteomics, metabolomics, genomics, etc. and assists in formulating individualized treatment plans for patients. AI also facilitates the process of drug development by interrogating biological data in order to find any relevant targets and pathways for diseases, which can also help to build new drugs or improve current ones. The study investigates the existing frontier of clinical information with emphasis on whether use of stem cells and AI in the treatment of Spinal Cord Injury (SCI) is close to being effective clinically, gives an overview of progress made in AI research on SCI.

In the end, this discusses several limitations, which should be pointed out when considering stem cell therapy for spinal cord injury (SCI). The primary issue stems from the fact that almost all the clinical studies had inconsistent and variable findings based on the type of stem cell, the timing of the intervention, the level of injury, and so forth. Contrary to rats, in humans, it has been difficult to apply such studies. Other factors such as rejection by the immune system, instability of the genome, and associated cancers make the application of stem cells in clinical practice much hard. There is also the consideration that since the stamping of stem cells is done in vitro for a long time, the stem cells may lose their potency due to senescence rendering the therapy ineffective [46-49]. Rehabilitation après spinal cord injury (SCI) is characterized by gradual and essentially laboratory-developed processes of creating new neuronal connections (relay mechanism), which does not suggest how long this will take in humans. In addition, ethical issues, regulatory restrictions, and difficulties in tailoring such treatments to each

individual patient slow down the development of stem cell-based therapies. There is an urgent need for further studies in order to improve these protocols and design individual therapies, in order to pave the way to clinical success in treating SCI.

III.Data Collection and Preparation

The data were extracted from the Singapore General Hospital (SGH), Singapore. The information obtained included gender, age factor, and spine injury cases due to stem cell development after serving the spine injury. There are two ways to work on the proposed datasets. We were expressed as an analysis of the machine learning classification techniques namely SVM proposed datasets train and image segmentation method with range of images frequency to highlight the stem cell in images. There were 3000 total CT scans of patients we used in this research, including 2090 female and 1020 male SCI cases, during the study period. Few cases were resolved due to minor or initial recovery once diagnosed, but many cases were reported for serious conditions and chances of paralysis for a lifetime due to SCI. In another part of this research, we used computational techniques to identify the cell culture and cell position using MSC stem cell images. The phase-contrast micrographs of monolayer cultures were used to build the image analysis method for classifying MSC phenotype. The method's overall flow is depicted in Figure 1, with each step being covered in detail in the sections that follow. The program estimates the number of cells after preprocessing. Cell-containing regions of the image are identified using thresholding and morphological techniques. To determine if the cell region is made up of a single cell or a cluster of cells, appropriate markers are placed within these regions. To identify individual cells, a cell cluster is further segmented. Following each cell's segmentation, the algorithm extracts a number of morphometric and textural properties that were designed by humans. The Image Processing Toolbox in Python software provides a complete range of reference-standard algorithms that are used in the development of cell segmentation and feature extraction methods. These features are used to train machine learning classifiers, which can differentiate amongst MSC phenotypes. Using Python 3.5.6 libraries, classification models were created in Jupyter Notebook 7.0.8.

IV.PROPOSED DATASETS

Mesenchymal stem cells (MSC) obtained from human bone marrow were seeded under standard conditions that were optimized for research on spinal cord injury (SCI) at a density of 100 cells/cm². On the second- and fourth day following preparation, the cultures were photographed to track changes in cellular phenotypes as the MSCs multiplied. This observation offered crucial information on the therapeutic potential of MSCs for the recovery of SCI patients. It was anticipated that by day 4, there would be about 6500 cells/cm², up from about 1000 cells/cm² on day 2. High-quality images of the cultures were taken using a Motic AE31 phase contrast microscope with a 10× objective lens and a Moticam 1SP 1.0 MP camera. Each image had dimensions of 1280 × 1024 pixels and a resolution of 1.56 pixels/μm. For the purpose of developing and testing algorithms intended to categorize MSC phenotypes, cell cultures and images acquisition were done three times. This resulted in an extensive dataset. After manually segmenting the cells using Python software, MSC cultivation and analysis were utilized to classify the cells into several phenotypes. While the third culture functioned as an independent testing set, images from two of the culture repeats were used as ground truth to train the algorithm and prepare the suggested datasets. A total of 472 cells from 100 photos made up the training dataset. These cells were all categorized into distinct phenotypes that are essential to comprehending MSC behavior in SCI. To make sure the technique is generalizable, validation was carried out using 50 micrographs totaling 190 cells, which were then further refined by a group of 20 trained persons. This dataset was created to facilitate studies on MSC behavior in the setting of spinal cord injury (SCI), with an emphasis on the cells' proliferative characteristics and capacity to differentiate into distinct cell types that could aid in spinal cord regeneration and repair. (Mota et.al, 2021). The images are taken in Singapore General Hospital (SGH), Singapore.

V. METHOD

This study leverages advanced technologies, including neuroimaging, behavioral data analysis, robotics, and artificial intelligence (AI), to develop an integrated framework for improving PD management. Parkinson's disease, as the second most common neurodegenerative disorder globally, affects approximately 8.5 million individuals worldwide and poses a growing public health and economic burden. Traditional clinical management of PD, heavily reliant on subjective assessments such as the Unified Parkinson's Disease Rating Scale (UPDRS), faces significant limitations, including inter-rater variability, low sensitivity, and restricted resolution. These challenges underscore the urgent need for innovative, objective assessment tools and personalized therapeutic strategies to address the dynamic and multifaceted nature of PD progression. The proposed Parkinson's Disease detection methodology fuses neuroimaging data-CT/PET with behavioral assessments and AI-driven insights to predict cognitive decline and motor deficits. High-resolution CT and PET scans are collected; for example, regions of interest include the substantia nigra and basal ganglia, along with longitudinal behavioral data such as motor performance, speech, and gait analysis, and demographic details. Preprocessing includes noise reduction, intensity normalization, and spatial alignment, while data augmentation with GANs will balance PD and control samples. AI-driven feature extraction will make use of CNNs for neuroimaging to analyze cortical thickness, dopaminergic

activity, and texture anomalies; temporal behavioral features include tremor frequency and memory errors. A multimodal neural network combining CNNs for image analyses and RNNs/Transformers for behavioral data integrates the two via a fusion layer for disease classification, with the prediction of cognitive or motor decline. Following this, rigorous internal validations-external validation with k-fold cross-validation is conducted, while explainable AI techniques such as Grad-CAM provide insights into critical brain regions. A robotic-assisted system allows for real-time monitoring of the patient, adaptation in rehabilitation, and feedback through AI-based predictions. While the cloud-based platform automates data processing, diagnostic reports, and personalized therapeutic suggestions to clinicians.

A. Patients and Methods

This study is a pragmatic, observational clinical trial carried out in the Department of Neurology and Neurosurgery at Singapore's National University Hospital between 2023 and 2024. The objective of the study was to study the clinical endpoints of patients with SCI who received stem cell therapy as an adjunct to standard surgical protocols. Prior to the study, ethical clearance was obtained from the hospital's institutional review board, and patients were informed in detail of the study objectives, their potential risks, and the measures they could undertake to minimize such risks.

Inclusion criteria were the following: patients aged between 25 and 60 years; the diagnosis of traumatic spinal cord injury with loss of motor and sensitive function below the injury site; lesions in the spinal cord verified radiologically; and eligibility for stem cell therapy based on neurological evaluation. Patients with progressive neurological disorders, active infections, previous spinal surgeries, and the history of malignancies or autoimmune diseases were excluded from the study.

A total of 3,000 patients were recruited for the trial from the department of neurosurgery and outpatient clinics using convenience sampling, consisting of 2,090 females and 1,020 males. All patients received some form of surgical decompression and stabilization procedure, along with mesenchymal stem cell transplantation. This was followed by artificial-intelligence-based imaging techniques to facilitate the precise detection and monitoring of stem cell activity post-transplantation. The information given by this AI method used real-time MRI and CT scan data that provided excellent scopes for accurate tracking of integration, differentiation, and tissue regeneration of the stem cells in the spinal cord through image detection, Identifying the Candidate Cell Region, area of fraction, and cell segmentation and validation method. Motor function, sensory recovery, and pain relief were evaluated using standardized neurological and functional scales at baseline, 3 months, 6 months, and 12 months post-treatment. The AI-based detection method also provided an exceptional insight into the therapeutic effects of stem cells and thus provided essential information for a comprehensive evaluation of recovery of the patients. The proposed design hybrid model is mentioned in Figure.2.

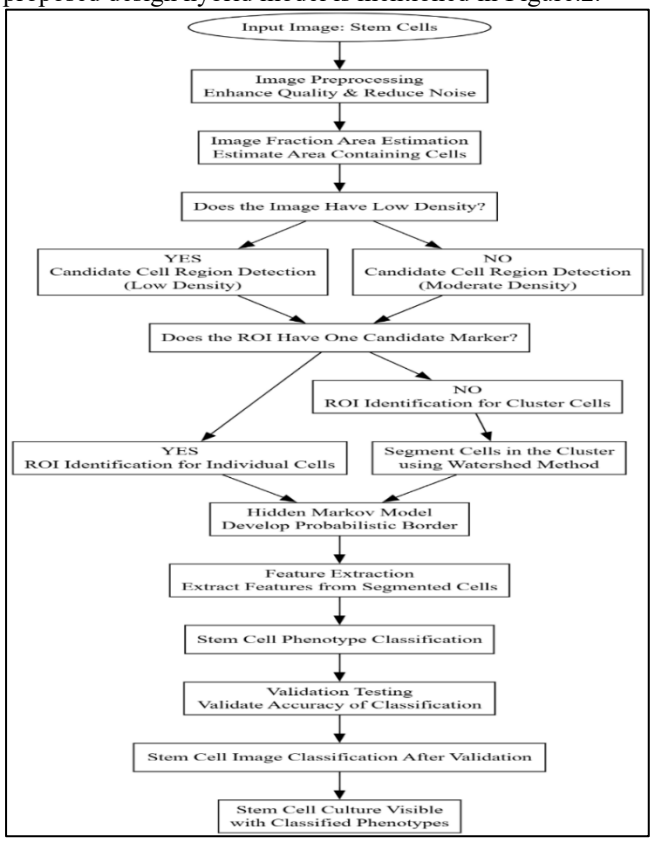
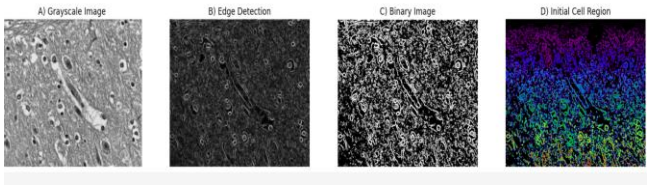


Fig. 2. Phase-contrast micrographs of SCI stem cells are classified using an algorithm pipeline. Examples of image densities include (a) low and (b) intermediate. Image (b)'s cell ROI comprises (c) single cells and (d) cell clusters, which are distinguished by the quantity of candidate markers (blue) within the ROI (white).

This is an enhanced hybrid technique directed towards the detection of stem cells. It improves accuracy and increases details on the redundancy and precision. It creates a new platform for both the medicine and the technology fields in that it allows for the estimation of the total number of stem cells produced postoperatively after the spine surgeries. Below is a brief explanation of the entire procedure of the five-step approach in the technique, which involves combining four methods to create this hybrid system, which will be explained below:

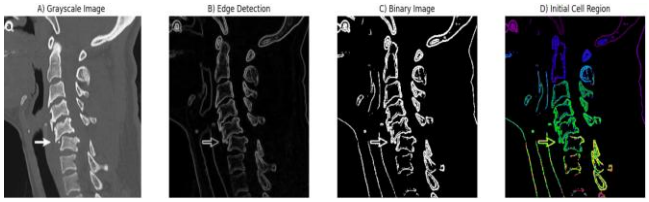
A. Image Optimization and Area Fraction Measurement

The RGB phase-contrast micrograph of spinal cord injury stem cells is preprocessed to lessen the impact of unwanted flaws created during imaging, and the resulting grayscale (Igray) image is displayed in Figure 3(a). In order to improve the intensity variation between the cell areas and the substrate and facilitate segmentation, the contrast of Igray is changed. Unsharp masking is used to sharpen the edges of the cells, and anisotropic median-diffusion is used to filter the results and get rid of undesirable artifacts while increasing the signal-to-background ratio without warping the edges. [Figure 3(b)]. Figure 3(c) shows the preprocessed image (Ipreprocessed) after having been subjected to a Sobel filter for the purpose of identifying cell borders. The Sobel operator detects edges above a sensitivity threshold of 1 and indicates regions with the highest intensity change. Dilation and closing are used to join the identified edges after object outlines have been produced. To eliminate any gaps in the filled areas, a flood-fill operation is then carried out. Segmentation performance suffers when morphological procedures are performed on images with a high number of cells and images with a low number of cells using the same structuring element sizes as mentioned in Figure 3(a) and Figure 3(b).



Day.1: Detected 346 cells in Image.1
Day.5: Detected 569 cells in image.1

Fig.3(a). Steps for MSCs Initialization Using Ant Colony Algorithm. (a.) Image of the input phase contrast microscopy is converted to the gray image and processed (b.) using steps such as contrast adjustment, sharpening, and anisotropic filtering. (c.) The resulting micrograph is segmented by detecting edges (d) that are connected and filled using dilation, closing, and flood-fill operation to obtain initial cell regions (Initial−region).



Day.1: Detected 278 cells in Image.2
Day.5: Detected 529 cells in image.2

Fig.3(b). The steps from the beginning of cell region detection show the MSC view: (a) The input phase-contrast micrograph is converted to grayscale; (b) contrast adjustment and sharpening and anisotropic filtering were applied for preprocessing. (c) Edges were detected; (d) edges were connected and filled by dilation, closing, and flood-fill operation to obtain an initial cell region (Initial-region).

As the culture increases over time, variations in the size and shape of the cells and clusters become even more important than their quantity. This issue is resolved by using area fraction (AF) as the determining factor for cell density, since distinct parameters are applied for low and moderate densities in order to distinguish between cell regions and the markers within them. Additionally, each image is assessed according to its density estimate rather than the possibly incorrect presumption that the length of time an image is in culture is a reliable indicator of density. The algorithm is automatically optimized so that it performs equivalently with respect to all ranges of cell density levels, according to the cell density-based criterion. The cell density in the input micrograph is derived using the AF of the binary image (Initial region). The algorithm now counts the number of white pixels in the image. The total number of stem cell detection is mentioned in the table below (Table 1).

An AF of 0.1 was used as a threshold for the algorithm to determine whether a picture is less dense (<0.1) or moderately dense (≥0.1) based on the training dataset. If an image has both low- and moderate-density parts, the algorithm will determine whether it is less or more moderately dense based on the region that is most prevalent. Greater AF and handling of the image as moderately dense are more likely to occur in an image with a bigger region of moderate density. In Figure 4 illustrates the various steps involved in locating possible cell regions. (a) Morphological and edge detection techniques discover the first input region. (b) Detected objects that are not cells

are eliminated by size, intensity, and shape rules. (c) The application of erosion and opening maximizes the area shape of the cell. (d) Final candidate cell areas are those found by getting rid of incomplete cells at the image border. The borders of cell regions are now indicated by blue highlights.

Since the thresholds for these photos were adjusted to enable sophisticated cell segmentation, it would be easy to identify the less-dense cells that were present. It should be noted that images with just less-dense cells would not benefit from the same thresholds used for moderately dense images, as this could result in more false detections. Conversely, a little cluster of cells would probably be found in regions of intermediate density in pictures that were categorized as less dense. The best thresholds for identifying markers in these clusters and separating individual cells are present in the low-density images. It shows from Figure 5 that, through incorporation of additional analyses, precision is ensured between cells and noncellular objects, assisting in the fine-tuning of region boundaries and achieving accurate feature extraction for biological studies. (a) Refined Markers Inside Cell in terms of Minute markers are used in the regions of consideration for locating the candidate cells on the basis of previously placed coordinates; also used as initial markers for distinguishing cells from non-cellular objects. (b) Operation isolates individual cells, with markers crossing each cell from the surrounding area, making them suitable for accurate feature extraction. (c) Identifies clusters of closely packed cells, highlighting the boundaries of regions in which further segmentation is required. (d) Segmentation algorithms successfully separate individual cells within clusters, with red outlines indicating boundaries drawn by the algorithm for further refinement of cell borders. (e) The finished output shows the refined boundaries of individual cells and clusters while excluding noncellular objects, thus allowing for accurate feature extraction for further biological analysis.

Table 1. Mesenchymal stem cell culture dataset

	For proposed Datasets.1		For proposed Datasets.2	
	Culture Day	**Total No. of Cell detection through image**	**Culture Day**	**Total No. of Cell detection through image**
Training Set	2	346	2	278
Culture SC1 and Culture SC-2(Datasets)	5	769	5	629
Independent testing	2	104	2	51
Culture SC- 3	5	129	5	112

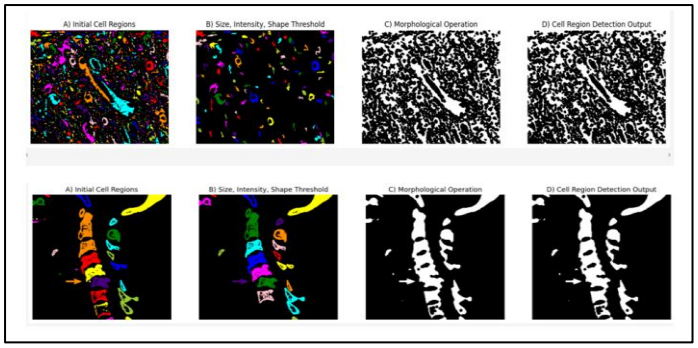


Fig. 4. Illustration showing the steps involved in identifying possible cell locations. (a) The morphological and edge identification techniques result in the discovery of the input region first. (b) Detected objects that are not cells are eliminated by thresholding based on size, intensity, and form criteria. (c) Erosion and opening maximize cell area shape. (d) The final candidate cell regions are obtained by removing incomplete cells from the image border. (Blue highlights are used to indicate cell region borders).

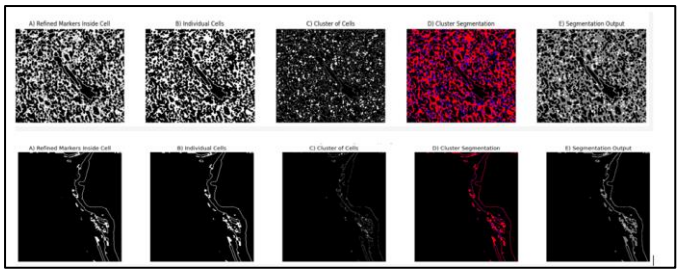


Fig.5. This image highlights the fine markers marked in all the region of a cell after the coordinates of the candidate cell location have been established. The markers are involved in further analysis, ensuring precision in distinguishing between cells and non-cellular objects, in refining the region boundary, and in assisting in accurate feature extraction for biological studies. (a) Refined Markers Inside Cell: Fine markers are placed within potential cell regions to mark candidate cell locations based on previously established coordinates, serving as starting points for distinguishing cells from non-cellular objects. (b) Individual Cells: The process isolates individual cells, with markers delineating each cell from surrounding areas, preparing them for precise feature extraction. (c) Cluster of Cells: Clusters of closely packed or overlapping cells are identified, highlighting the boundaries of these regions where further segmentation is required. (d) Cluster Segmentation: Segmentation algorithms separate individual cells within clusters, with red outlines indicating distinct boundaries to refine cell borders. (e) Segmentation Output: The final output shows fully refined boundaries for individual cells and clusters, excluding non-cellular objects, enabling accurate feature extraction for further biological analysis.

B. Identifying the Candidate Cell Region

The cell area may comprise several cells since the actual cell region recognition is done semantically to identify pixels belonging to cells as said in the truth. The entire procedure of candidate cell region detection is conveyed in Figure 6. I_initial region directly defines regions with probable cell objects in images that are less dense. So, each object is thresholder [Fig. 6(b)] and undergoes morphological operations [Fig. 6(c)] to distinguish picture artifacts from possible cell regions. Preprocessing is designed to find objects with high sensitivity [Fig. 6(a)]. Size thresholding is used to eliminate an object if its area is below a predefined threshold, which is determined by averaging, plus or minus one standard deviation, or calculating the mean or minimum of all foreground objects' areas in the image. This ensures that the threshold values are not heavily skewed toward the training set since this adaptive method considers the relative sizes of the objects themselves in each image to determine whether or not they are likely to be cells. Similarly, for the intensity-based thresholds, the maximum and minimum intensity values within the object obtained from the pixel coordinates in Ipreprocessed are applied. After contrast enhancement, objects in the training set labeled as cells showed bright cytoplasmic pixels and/or dark nucleus interior pixels, as shown in Figure 4. If both features are absent, this would indicate that the object is not a cell because its intensity would tend to be similar to that of the substrate, not to mention quite homogenous. Shape-based thresholding takes into account circularity and ellipticity of the object. From training data, the circularity of MSCs was confirmed, and that of bright-phase imaging artifacts was excluded. The ellipticity of MSCs was determined to be extremely thin for artifacts like fibers or strands where 1 is a line segment and 0 is a circle. Therefore, circular objects and elliptical objects were removed from the cell regions detected, as MSCs are less circular or elliptical than the artifacts. After thresholding, morphological processes like opening and erosion are used to fine-tune boundaries. In order to prevent the analysis of truncated cells, objects whose pixels are connected to the image border are finally eliminated. All of these steps result in the final image (Icell-region), which corresponds to regions of interest (ROI), as Fig. 6(d) illustrates. The edge detection stage covers all cell edges in moderately dense images by using a 0.5 sensitivity threshold. Different sizes of structural elements are used for dilation and closing in order to obtain new possible cell areas. This method of thresholding is used with similar threshold values as for images with fewer objects, but with different threshold values for images with many objects. In low cell density processing, thresholding is also repeated more often, since a lower threshold can lead to false detections. Moderate cell density is followed by thresholding, morphological opening, and morphological closing.

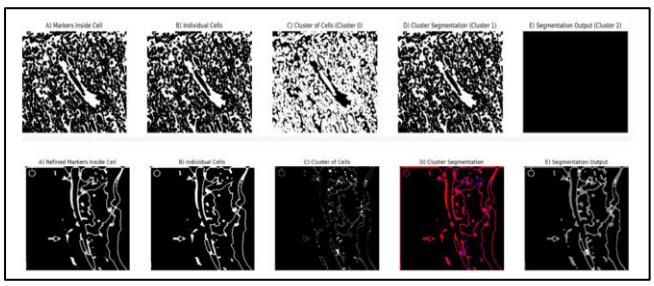


Fig.6. Pipeline for segmenting markers from the I_cell-region inside the regions of interest (black and pink). (a) Ipreprocessed is processed to create an image that is ready to be used as a marker (I_marker-treated). (b) The H-minima transform with a larger threshold A is used to obtain markers from I_marker-processed data. (c) Using the H-minima transform and a lower threshold B, markers are obtained from I_marker-processed data. (d) For ROIs that meet the perimeter threshold requirement as well as for ROIs from threshold A that have zero markers, markers from both thresholds are combined.

C. Identifying Candidate Markers

In Phase-contrast micrographs of stem cells from spinal cord injuries show that the intensity is highest around the cell boundaries. This is due to the optical path difference (refractive index and substrate thickness) between the cells, and the substrate maximizes the phase shift at these locations. The relative homogeneity inside the cell causes the intensity to be lower. Candidate cell markers are the darkest areas within a cell; they are used to separate individual cells, especially in clusters, because each cell usually has one noticeable regional minimum. The image I_preprocessed_{\text{preprocessed}} Ipreprocessed is processed using Gaussian and median filtering to eliminate noise and misleading local minima that don't match real markers in order to improve the image for better marker detection. The contrast of the regional minimum is then enhanced by applying contrast-limited adaptive histogram equalization. In order to get I_marker-I_processed_{\text{marker-processed}} I_marker-processed, morphological reconstruction is finally carried out utilizing the histogram-equalized image and I_cell-I_region_{\text{cell-region}} I_cell-region as the mask, as shown in Figure 6(a).

Two threshold values, A and B, are used to segment markers for the H-minima transform. If the lower threshold value B is used, it may result in false positives (FPs), while using the larger threshold value A alone leads to under-detection of markers. As a result, the two criteria are integrated. In order to locate possible markers inside the regions of interest (ROIs) from Icell-I_region{\text{cell-region}} I_cell-region, a high value for threshold A is first used. As seen in Fig. 6(b), morphological opening and binary area opening are used to eliminate non-marker objects. The lower threshold value B is then used to obtain minima. As we can see that in Figure 6(c), these minima are dilated and closed to form the candidate markers. The markers found with threshold B are added to the region if no markers are found using threshold A in any ROI. As seen in Fig. 6(d), markers from both thresholds are combined in prospective cluster ROIs using perimeter as a criterion. Area thresholding, morphological procedures, and distance thresholding are done in regions with several markers to solve over-detection problems. A cell region's centroids are measured for the Euclidean distance; if this distance is too small, the region becomes over-segmented, and the marker with the smaller area is eliminated. The last step involves dilation and erosion to get rid of any last over-segmentation.

D. Cell Segmentation and Validation

To identify and demarcate every cell in the image, the ROIs were merged with markers as shown in Fig. 7(a). One marker identifies one cell [Fig.7(b)], several markers identify a cluster of cells [Fig. 7(c)], and an area is left unmarked. The number of cells in each cluster corresponds to the number of markers in that cluster. Marker-controlled watershed then divides the region into distinct cells for each DC, thus ameliorating the inherent drawbacks of the conventional watershed approach, such as over- or under-segmentation [Fig. 7(d)].

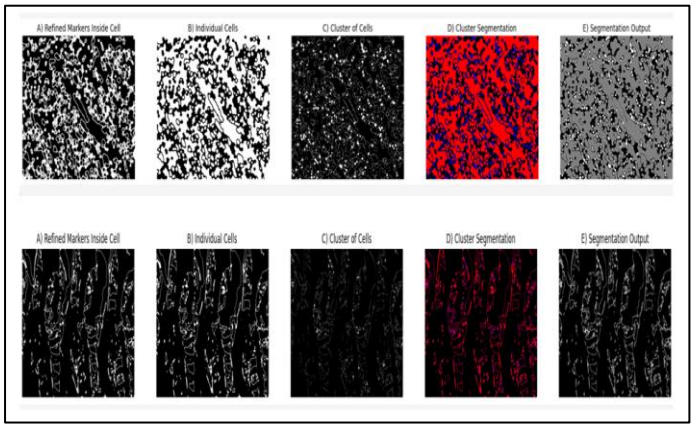


Fig.7. The steps involved in cell segmentation are represented. (a) The findings of marker detection are combined with the I_cell-region (blue markers are displayed). ROIs with one marker are recognized as individual cells in (b), and ROIs with several markers are recognized as cell clusters in (c). (d) Watershed segmentation inside clusters is done using marker-controlled watershed (red watershed ridge lines). (e) The segmentation output is obtained by combining the results of stages (b) and (d).

E. Feature Extraction

To categorize stem cells into different phenotypes, automatically derived human-engineered descriptors were used. Size, shape, and statistical texture characteristics were among the thirty features in total that were computed. The purpose of these characteristics was to distinguish between flattened and spindle-shaped stem cells. While texture-based features used spatial intensity distribution to distinguish flattened stem cells from those with a large phase-contrast halo, morphometric features were used to identify spindle-shaped cells. Segmented cell areas were used to compute first-order features, while the gray-level co-occurrence matrix (GLCM) was used to assess second-order features. Each second-order feature had 50 measurements since the GLCM features were calculated in 24 locations. The region of curve (ROC), area under the curve (AUC), and 95% Confidence of interval are used to choose a single metric for cell type discrimination. To maximize classification accuracy, the top 30 features were sorted by AUC, and features with high correlation (>0.8) were eliminated. Table 2 depicts a deep comparison of the dataset used for stem cell culture and feature extraction and classification. Here are two datasets, suggested as Proposed-1 and Proposed-2, which, on the days of culture, constitute the basis for separation of the training and independent testing sets analyzed here. In specialist terms, features such as the days of culture and total number of cells detected through imaging are included in this dataset in the training and testing sets. For the training set, on Day 2, Proposed-1 includes 842 cells detected through imaging while Proposed-2 includes 656 cells. Coming on to Culture SC 1 and Culture SC 2 datasets, where, for Day 5, Proposed-1 has 1538 cells detected, while Proposed-2 rolls with 1358 cells which is mentioned in Table 2.

Table.2. SCI stem cell culture dataset used for feature extraction and classification

.

	For sample proposed-1		For proposed Datasets-2	
	Culture Day	**Total No. of Cell detection through image**	**Culture Day**	**Total No. of Cell detection through image**
Training Set	2	842	2	656
Culture SC 1 and Culture SC 2(Datasets)	5	1538	5	1358
Independent testing	2	210	2	119
Culture SC 3 for images	5	358	5	324

F. Cell Classification and Validation

We use a sequential approach to model the dynamic process of stem cell formation, followed by classification and validation of stem cells altered by spinal cord injuries with the use of a hidden Markov represent (HMM). The HMM captures the probabilistic temporal transitions between various states of stem cell progression during spinal cord recovery. The HMM was set up such that states of spinal cord injury stem cell improvement were indicative of particular stages of development of stem cells, such as undifferentiated cells, intermediate states, and fully differentiated cells, for classification purposes. Every state reflects a unique step in the restoration process. The sequence of observations comprises quantifiable features such as changes in cell shape, changes in gene expression, or functional properties. These attributes act as "observations" in the HMM, assisting in inferring the development phase or hidden state of the cells. The HMM parameters are set using emission probabilities that define the likelihood of an observation given a state, transition probabilities that denote a transition from one state (e.g., undifferentiated) to another (e.g., differentiated), and initial state probabilities that define how the initial states are distributed.

HMM is trained using labeled data obtained from experiments or from known cell development stages (e.g., pluripotent and differentiated). The existing transition and emission probabilities are adjusted based on the observed sequence likelihood using the Baum-Welch algorithm, an expectation-maximization method that computes a suitable set of statistics, known as summarization variables, in order to maximize the probability of the observed sequences. For classifying, the forward method is utilized to obtain the likelihood of being in a stem cell state, given its observed attributes over the entire period of progress, making it more straightforward to predict which state, for example, stem cell development phase, is most likely at any point in time.

Moreover, Viterbi algorithm is employed to pinpoint the most probable sequence of the hidden states (or cell maturation phases) of a sequence of observations, capturing the gradual state transitions which occur in stem cells. In order to guarantee that the model generalizes well to new data, validation is performed using validation methods including k-fold validation. A part of the data is used for training and the remaining data is used to evaluate the model's classification accuracy. ROC curves are plotted to check the model's performance, and the AUC (Area Under the Curve) is computed in order to measure how well the model distinguishes between different stem cell improvement stages whereas the 95% CI indicates the range of cell detection. Feature selection is the process of

pre-selecting attributes for classification which are significant discriminants, for example according to their AUC, to further refine the model and enhance its accuracy and efficiency as shown in Figure 9. Furthermore, parameter tuning is conducted through tuning the HMM parameters (transition and emission probabilities) based on validation results to improve the classification performance. The model can be applied for continuous monitoring of stem cell recovery for spinal cord injury and also for classification of new stem cell data once the model has been trained and validated. HMM is a powerful technique to analyze how stem cells travel back and forth between stages over time, promoting tissue regeneration and functional recovery which is represented in Figure 8.

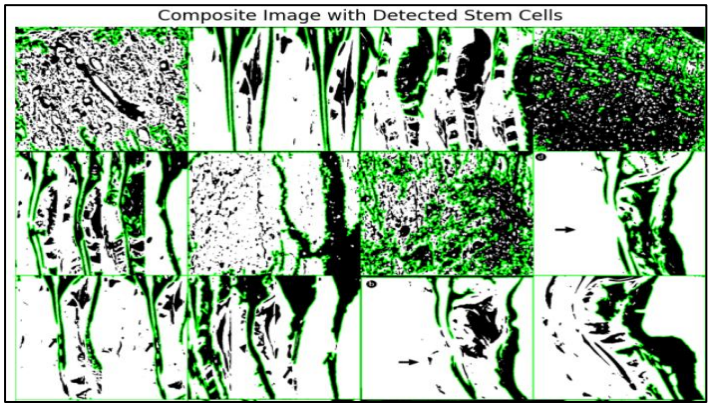


Fig.8. The following steps encompass the entire paradigm of cell segmentation through Hidden Markov Programming-based segmentation: a) It shows the blue markers in the I_cell-region by combining the detection of initial markers. b) ROIs consist of the single marker and are recognized as cells individually. c) Recognizes ROIs with multiple markers as clusters of cells. d) Inside the clusters, it uses marker-controlled watershed segmentation, red watershed ridge lines indicating boundaries. e) Output from the final segmentation is due to observations in (b) and observations inside the clustered region in (d).

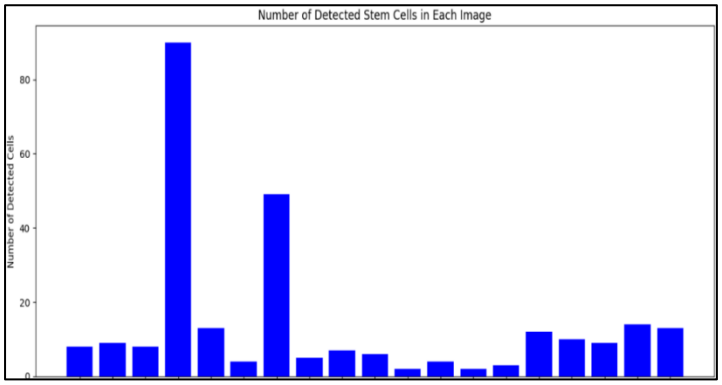


Fig.9. Graphical representation of segmented stem cell detection in the proposed images

VI. RESULTS

The algorithm evaluated detects and segments spine injury stem cells, specifically the MSCs through various evaluation criterions. It evaluated the algorithm based on the data set for SCl stem cells through sensitivity and precision. For the training set, the algorithm thus achieved a sensitivity is 0.99, thus indicating worthwhile performance. Furthermore, the precision of the algorithm appeared to exceed 0.98 in the training set and 0.99 for independent testing of cell culture. The AI based algorithm performed better than the conventional U-Net model, especially for cluster cell detection and segmentation due to the marker-controlled watershed approach as opposed to U-Net's weighted map approach as shown in Figure 8. Both algorithms were indeed trained with different parameters for low and moderate cell densities with the algorithm demonstrating more than a reasonably good performance for both cases: no significant observed difference for sensitivity, precision, or F1-scores. A paired two-sample t-test statistically affirmed that the algorithm greatly outperformed U-net in detecting and segmenting SCI cells. The algorithm is very efficient in detecting and segmenting SCI stem cells compared to existing methods, especially in separating clustered, stem-like cells which are mentioned in Table 3 and performance evaluation of sensitivity, specificity, accuracy, precision, and F1-Score of the algorithm for training and independent testing is mentioned in Table 4.

Table. 3. HMM state represents the stem cell availability

Not Stem Cell	1.00	1.00	1.00	8
Stem Cell	1.00	1.00	1.00	11
Macro Avg	1.00	1.00	1.00	19
Weighted Avg	1.00	1.00	1.00	19

Table.4. Cell detection for Performance Metrics in terms of sensitivity, specificity, accuracy, precision, and F1-Score of the algorithm for training and independent testing

	SCI Stem cell	**Correct Detection True Positive**	**False Detection True Negative**	**Under Detection False Positive**	**Mislead Detection False Negative**	**Sensitivity**	**Specificity**	**F-Score**	**Accuracy**	**Precision**
Training	All	596	3	2	3	0.99	0.987	0.99	0.99	0.99
Independent testing	All	899	1	0	6	0.99	0.988	0.99	0.99	0.98

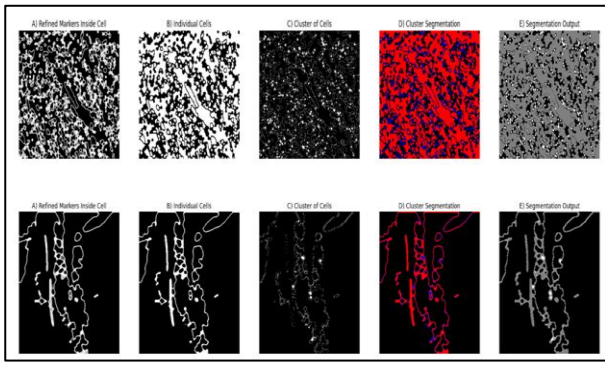
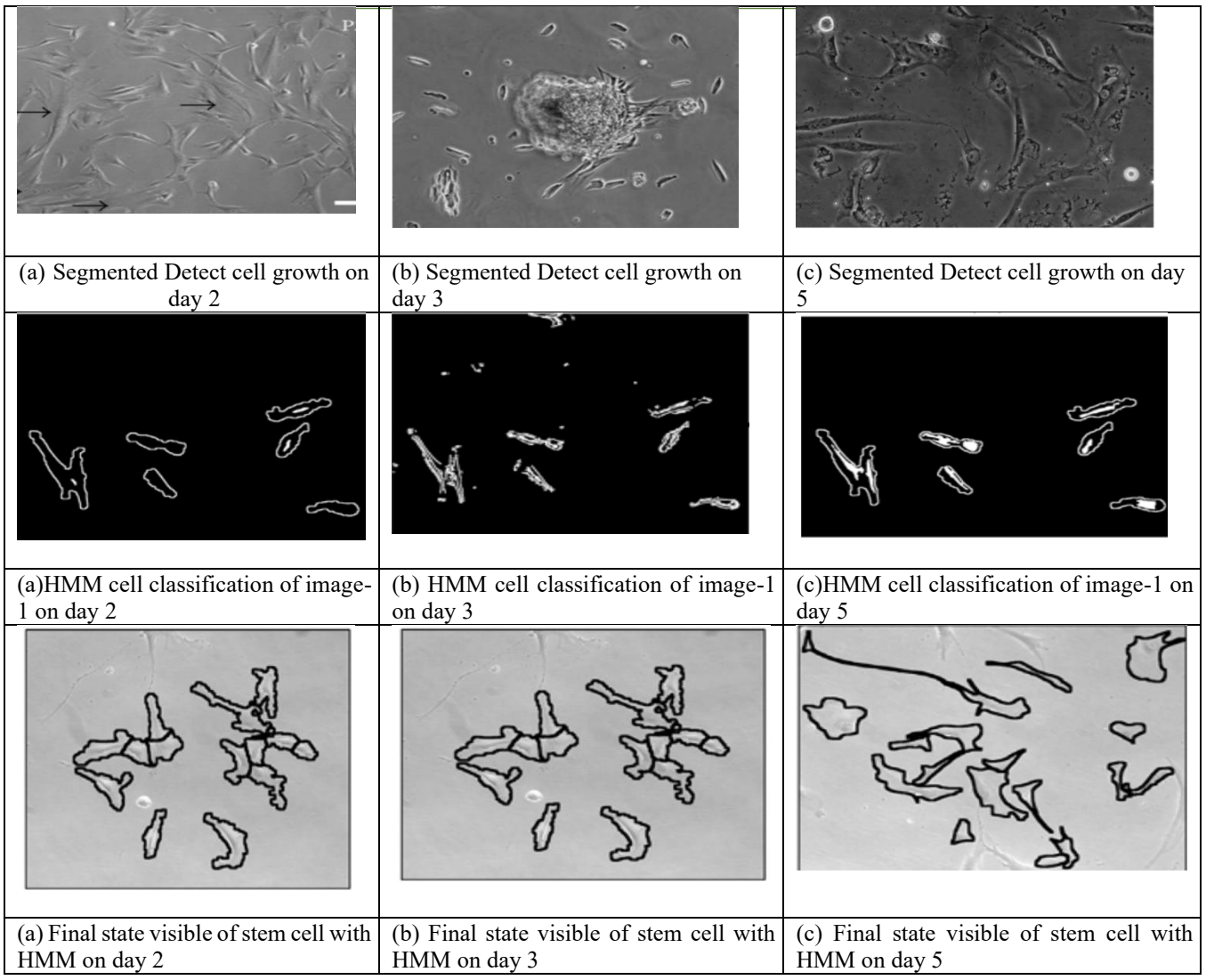


Fig. 8. shows the ground truth along with algorithm's segmentation results for two images of both low and moderate density from the test dataset. The difference in performance of the algorithm to localize cells in low and moderately dense images was also reviewed as it was trained with different parameters for these two levels of cell densities.

A. Hidden Markov Model (HMM) Application in Cell Segmentation

In this section, we call upon an independent test set image that demonstrates our system, where the first row shows the true cell outlines, while the last shows segmentation results using an algorithm with HMM. The first two columns show low-density cells where the matching cell outlines labeled by the algorithm resulted in a small discrepancy between the true and predicted outlines. Similarly, columns (c) and (d) show moderate-density cells, and the algorithm effectively captured the cell outlines. Such segmentation results are refined by using the HMM in this case to promote training of the algorithm in recognizing patterns of cell distribution in the image space, enabling efficient classification and segmentation, whether they represent poorly distributed or moderately distributed cells. Additionally, ground truth images have been processed following standard practices, which involve ignoring cells truncated along edges of the image, ensuring that segmentation only involves complete cells in the analysis as shown in Figure 9 and the total no. of stem cell detection accuracy is mentioned in Table 5.



(a) Segmented Detect cell growth on day 2 | (b) Segmented Detect cell growth on day 3 | (c) Segmented Detect cell growth on day 5

(a)HMM cell classification of image-1 on day 2 | (b) HMM cell classification of image-1 on day 3 | (c)HMM cell classification of image-1 on day 5

(a) Final state visible of stem cell with HMM on day 2 | (b) Final state visible of stem cell with HMM on day 3 | (c) Final state visible of stem cell with HMM on day 5

Fig.9. For instance, this is the first comparative dataset containing images of MSCs from an independent verification test dataset juxtaposed against the original outlines of the cells (first row) with the algorithm segmentation results (last row). Column (a) and (b) show pictures of chi manipulated by the algorithm in low density which have coincided cell outlines. These were given AF values of 0.06 and 0.11 when estimated by the algorithm. Likewise, column (c) and (d) provided images of moderate density containing images with AF values 0.18 and 0.29 also found respectively. Indeed, ground truth images are consistent with the procedures such that any cells cut off along picture edges are not included in the analysis.

Table. 5. Cell detection for Performance Metrics in terms of sensitivity, specificity, accuracy, precision, and P- values of the algorithm for training and independent testing

	Total No of Cells Detection	**Sensitivity**	**Specificity**	**Accuracy**	**Precision**	**P-Values**
Training	596	0.91	0.887	0.99	0.88	N/A
Independent testing	899	0.97	0.988	0.99	0.79	N/A

B. Stem Cell Classification

As is mentioned in the previous section, only successfully detected individual cells and cells in clusters with accurate counts were used in training and testing for identification of the cell phenotype with machine learning

models. Models included k-mean clustering, U-Net, and HMM which were trained and evaluated using five-fold cross-validation with AUC as the performance metric. Validation of the five classifiers trained using the object features from both the day-2 and day-5 cultures occurred through testing their performance in cross-validation for a combination of cells from day 2+day 5 and day 2 and day 5 independently. The day 2+day 5 models performed comparatively worse based on the day-2 test set compared to the day-5 test set. This bias might arise from the model's fitting given the predominance of day-5 MSC features. Training the classifiers with features from day 2 and day 5 individually enhanced performance for each of the days. The outputs of the two classifiers with maximum classification area under the receiver operating characteristic curve for each day were for days 2 and 5 k-mean clustering, U-Net, and HMM. The classification agreement plot between the models used for ensemble classification is shown in Figure 7. The x-axis of Fig.7(b) is not continuous because HMM's predicted probabilities are a discrete distribution. The different estimates given by the two best classifiers for each day may be due to their unique intrinsic learning from the same features. The k-mean clustering, U-Net +HMM models can capitalize on the advantages of a combination of linear and non-linear approaches to yield more accurate predictions. Such a scenario likely paves the way for the enhanced performance of fusion classifiers as compared to a stand-alone classifier during five-fold cross-validation, thus enabling their selection for the image analysis pipeline. The ensemble classifiers selected for day 2 and day 5 were further validated on an independent test dataset. The algorithm was, day 2 AUC = 0.98, day 5 AUC = 0.99, able to identify cell phenotypes correctly. The classification models for the two respective days proved to have gained above random guesses at statistical significance with 99%. In the end, Table 6 shows the comparison of the previous authors results and our proposed results.

Table. 6. Comparison of algorithm cell detection and segmentation performance

	Authors	Year	Total No of stem cell detection	Sensitivity	Specificity	Precision	95% CI	AUC	p-value
Training (Conventional method)	Sakina *et.al*, (2021)	2021	472	0.765	Not mention	0.653	95%	0.81	NA
Independent testing			186	0.747	Not mention	0.556	97%	0.78	NA
Training (HMM)	Our Proposed work	2024	596	0.91	0.887	0.99	97%	0.98	NA
Independent testing			899	0.97	0.988	0.98	98%	0.99	NA

VII. DISCUSSION

This image analysis technique is segmentation and classification of mesenchymal stem cells (MSCs) according to their cell morphological phenotype. Segmentation leads to a number of cells per image, cell density (cells per cm^2) and the percent confluency of the well, which gives an indication of growth characteristics of the culture. Also, the classification of the segmented cells gives a cell exclusion or unwanted fraction (i.e., non-viable cells compared to viable cells) for assessing culture quality. The high sensitivity, precision, and F1-score performance of the detection of MSCs in phase-contrast micrographs indicate that automated quantitative evaluation can integrate seamlessly into the current workflow in cell cultures. One of the important details to emphasize here is that the considered image analysis method has not been investigated in its entirety. Some phases of the method were tested only separately, with the use of reprocessing the previously obtained dataset. The proposed method should be tested in an extensive way, using a different independent dataset, in order to give an overall evaluation. The cell detection and segmentation depend on the original phase-contrast micrographs of the cell culture. The segmentation of the cells also depends on the quality of this stage. The classification of the cells also depends on the result of the segmentation stage. For that, refining is necessary to obtain the best result of the classification process. Work should be carried out in the future, to improve the method, and it should be tested using other datasets. The final stage of the work indicated some limitations, and possibilities to improve each one of the presented steps. This capability of the algorithm target cell detection and cell segmentation, thus quality endowed images need to be acquired. Among various factors, procuring images with sufficient high contrast is a prerequisite for phase-contrast micrographs to bring out cells from the substrate. The majority of detection errors suffered both during training and consumed as external testing were due to either low image contrast or blurring in certain regions. Higher contrast and more straightforward segmentation could be obtained with labels prepared for fluorescence microscopy, though it remains the standard technique for

performing observations on live, non-invasively. The results from classification were analyzed for the factors influencing the performance of proposed models. In addition, phenotype labels were obtained for the entire dataset from 50 trained individuals with varying expertise in culturing MSCs to assess the subjectivity of human classification and the generalizability of the algorithm. This enabled analysis of how ambiguity in cell morphology impacts visual inspection and classification.

The existing binary classifier may be fine-tuned towards the identification of indeterminate phenotype cells in a bid to reduce misclassifications and instill certainty in predictions. Further data collection would be required in training a machine-learning model to fit indeterminate predictions. Another approach would be to tune a binary classifier by calibrating its continuous probability output to reflect confidence in a given cell's phenotype. Future work will focus on fine-tuning the current approach by deploying such machine learning methods to boost classification robustness. Here, the dataset included images from three distinct cell populations. The method can be improved and validated on a more extensive collection of images from MSC cultures made in different laboratories and using various phase-contrast microscopes. Using additional data from other cultures may allow for sufficient features even for earlier time points, changing the way classifiers are used and making it unnecessary to have separate classifiers. A larger number of datasets will also help in increasing variability for the purpose of algorithm training, and it will also help in predicting the quality of the culture in an actual system. The center of gravity of the research conducted has been to show the feasibility of image-based analysis for assessing the MSC phenotype in a non-invasive and objective way in cultures of low and moderate densities. The application is not anticipated to be efficient in dealing with images of cells whose orientation is highly random due to overcrowding as this makes even visual examination difficult. Quantitative assessment of the earlier stages of the culture is more important for controlling wellbeing of the culture as MSC cultures are usually collected or subcultured before achieving high density.

VIII. CONCLUSIONS

This approach presents a remarkable opportunity for growth and development, such as the estimation of their percentage in the future culture based on previous culture data, the simulation of the effects of change in culture protocols, and the tracking ratio of non-functional to putatively effective MSCs as a function of time, confluence and cell density, etc. In addition, it can be used to evaluate the feasibility of other types of stem cell cultures as cytotherapies by means of image-based analysis of their morphology. The presented analysis has segmented and classified MSCs according to their morphological phenotype which enabled the non-destructive evaluation of the viability of cells in a monolayer culture. It also provides a link to monitor and control culture conditions throughout the experiment, it increases reproducibility of the treatment. The imaging solution that has been proposed will provide an efficient way to manage MSCs' quality without involving tedious manual procedures that would, otherwise, delay provision of effective stem cell therapies for chronic diseases.

A. Future Recommendations

The model for detection and classification of mesenchymal stem cells (MSCs) from the phase contrast images, as envisaged by us, promises further extensive scope for research on stem cells relative to spinal cord injury. However, there is much possibility left for its enhancement in utility. Tests with diverse and independent datasets are needed for the evaluation of the model's overall performance as well as its generalizability. Enhancement of image quality and contrast with more sophisticated methodologies, such as fluorescence microscopy, will bring a reduction in detection errors, as a consequence of an improvement in image quality itself. This in turn will benefit the segmentation and classification tasks by using data from the various labs and thus more extensive coverage of the diversity of the MSC populations. Solving the newest problem of high-density cell populations later in culture also requires the construction of intelligent algorithms to manage this scenario well. The model can assist neurosurgeons very much to identify the stages of stem cell increase during spinal cord injury treatment. This real-time image-based analysis on live MSC development will assist in timing the intervention and determining the validity of the intervention. Monitoring early-stage culture will therefore enable one to have stem cells of good quality ready for treatment, thus laying a good groundwork for this repair competition. Increasing the diversity of the dataset should also assist the model in generalizing on diverse experimental conditions, thus serving as a valuable tool for predicting stem cell growth and therapeutic efficacy. Further enhancement in this direction will immensely advance spinal cord injury and related stem cell applications in neurosurgery, thus accelerating the generation of better therapies.

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Data Availability

Data is available with the corresponding author and can be provided on request.

Author Contribution

Author contributions S.S.; Conceptualisation, Design, Writing, Reviewing, Analysing, Data Collection, Editing, Proofreading. M.Q.; Conceptualisation, Development, Writing Draft, Formal Analysis, Data Collection, Editing, Visualisation, Proofreading. NZJ.; Conceptualisation, Project Supervision, Editing, Visualisation, Proofreading. N.A.; Writing Draft, Visualisation, Data Collection, revising manuscript, editing. S.S.; Writing Draft, Reviewing, Visualisation, Data Collection, revising manuscript, editing. S.S.; Writing Draft, Visualisation, Data Collection, revising manuscript, editing. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declarations

Ethical Approval

This study was awarded an IRB approval by the Medicare Cardiac and General Hospital (MGCH) review board. It was conducted strictly in line with the Declaration of Helsinki principles, emphasizing the protection of human subjects in clinical and medical research. Compliant with the Declaration, the research relied on IRB approvals detailing the most appropriate manner through which to obtain informed consent from each subject in conformity with the ethics guidelines and federal regulations. Further, privacy and confidentiality were also afforded maximum concern; all regarding the subjects' personal information would be ensured and utilized only for the research intention. Informed consent to participate. For the purpose of this study, informed consent was not required under the authorizing IRB. for the aforementioned hospital-based school program, informed consents were obtained by the program from the legal guardians of the patients.

Consent for Publication Participants.

Consent for publication was given by all participants

Competing Interests

The authors declare no competing interests.

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