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Assessing the Safety and Efficacy of a Biotherapeutic Product for Treating Wounds Using Homologous Animal Model

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Abstract

Proliferation, inflammation, and tissue remodeling are stages in the intricate biological process of wound healing. The assurance of autologous cell therapy, which uses a patient's own cells to stimulate tissue regeneration and improve healing outcomes has been brought to light by recent developments in regenerative medicine. This study used chickens as a preclinical homologous wound healing model to assess a new autologous cell treatment technique. Chicken's autologous peripheral blood-derived mononuclear cells were separated and their appearance and functional characteristics were examined. Their migratory capacity a crucial component for tissue regeneration and wound healing was evaluated using a scratch test. Cell survival and traits similar to human therapeutic cells were further verified by functional and phenotypic investigations.

Each bird had excision wounds made in order to assess the effectiveness of the treatment; one wound was treated with the autologous cell preparation, and the other wound was left untreated as an internal control. Over the course of ten days, the progress of wound healing was tracked. With an average wound area reduction of 92% by day 10, the treated wounds showed noticeably faster healing than the untreated control wounds, which showed a reduction of 56.75% ($p = 0.019$).

Because of their similar immunological and healing systems, the study emphasizes chickens as a viable, moral, and economical substitute. This study demonstrates that the novel autologous cell therapy is both safe and effective in a homologous chicken wound model. It also highlights the affordability and translational applicability behind using chicken model for assessing cell-based therapies with the potential of autologous cell therapy to promote tissue regeneration and better healing outcomes.

Keywords: Wound healing, Autologous cell therapy, Process validation, Chicken model

1. Introduction

Wound healing is a complex process that involves various cellular mechanisms like inflammation, tissue proliferation, and remodeling. Minor wounds usually heal on their own without any rigorous treatments whereas chronic wounds, such as diabetic foot ulcers, post-traumatic ulcers, and venous leg ulcers do not heal through the normal healing phases. This reduced healing and deep wounds can lead to serious complications like infections, leg amputations, and long-term hospitalizations which places a significant burden on the Indian healthcare systems and the economy.

An innovative therapeutic approach called Autologous Cell Therapy (ACT) involves using donor's cells

after they have been grown and treated outside of their body. This method has the advantage of reducing the risk of systemic immunological reactions, bio incompatibility, and disease transmission linked to grafts or cells that were not grown from the patient.

This type of therapy has been effectively applied to treat burns and ulcers, promote wound healing, reduce chronic inflammation, bioengineer skin grafts, and enhance postoperative healing. [1]

As healthcare professionals study the possible benefits and drawbacks of cell-based therapy, significant concerns regarding safety issues and clinical study between the human and animal models have emerged in the area of regenerative medicine, which is why studies are progressing quickly to be applied in clinical settings. Understanding the entire range of cell functions as well as preclinical data for therapeutic efficacy and safety is essential. [2] The use of animal models to obtain this data has significantly expanded. New animal models are needed to be researched to broaden the scope of existing research.

Most of the studies have been conducted on rodents. Even while the current models provide insightful information, they are not suitable for many illness types and may not have the same size or physiology as humans. [3] Current animal models are weak in NK cells and lack functioning T and B cells. Their translational precision is limited by their tiny size and the ways in which they vary from humans in terms of metabolic and immunological signaling. [4]

Pigs, non-human primates (NHPs), rabbits, and dogs are examples of large animal models that are being used as models to mitigate the limitations mentioned previously with the existing animal models. NHPs are useful models for immune system research and gene therapy, whereas pigs are frequently researched for skin grafts, cardiovascular cell therapy, and organ transplants because of their anatomical resemblance with humans .[5][6] Rabbits are studied for tissue engineering and cartilage repair [7], whereas dogs are used for bone marrow transplantation and the study of neurological diseases.[8] Long lifespans, genetic diversity, high maintenance expenses, and ethical issues are a few of the major drawbacks of these models, which can make large-scale research difficult and resource-intensive.[9]

Avian species, especially chickens have demonstrated themselves to be an important commercial resource due to low cost as well as a vital resource for scientific research. A vaccine against Marek's disease virus (MDV- an oncogenic virus) was first developed for chickens. This vaccine is currently used extensively in large-scale industrial settings. Furthermore, the chicken egg has long been employed as an "incubator" in the manufacturing of human vaccines, including those against influenza.[10] Even though rodents are frequently used in preclinical research, chickens provide particular advantages for process validation.

They are considered valuable model due to their quicker wound healing compared to mammals and similar tissue repair mechanisms to humans, making them useful for translational research. Published studies have shown their application in tissue regeneration and dermatological research, allowing researches and healthcare professionals to bridge findings between laboratory experiments and human clinical studies. Additionally, chicken models offer low cost and less ethical burden compared to traditional mammalian models. Despite being evolutionarily distant from humans, chickens share many biological similarities with humans. For example: the discovery of the Rous Sarcoma Virus and the oncogene src in chicken laid the foundation for cancer research. Similarly, avian leukosis viruses (ALVs), which resemble human oncogenic retroviruses, have played a major role in the advancement of retrovirology research.

Many chicken genes have direct human homologs, with conserved sequences and regulatory elements. The fundamental immune mechanisms in chickens and humans are similar, including the presence of T-cells and B-cells. The distinction between these immune cells was first discovered in chickens, with B-cells named after the avian bursa of Fabricius. Many regulatory mechanisms, such as steroid hormone

signaling, were first studied in chickens. Chicken red blood cells retain their nuclei, making them an essential model for chromatin and gene regulation studies, contributing to understanding epigenetic modifications in humans.[11]

There are significant parallels between the immune systems of humans and chickens. While B cell maturation takes place in the bursa of Fabricius in chickens and in bone marrow in humans, both rely on bone marrow for hematopoiesis and a thymus for T cell formation. In both animals, immune responses are supported by secondary lymphoid organs like the spleen and MALT. Granulocytes, dendritic cells, macrophages, NK cells, and T and B lymphocytes are among the immune cells that they have in common. Both employ phagocytosis, cytokine signaling, and PRRs (such as TLRs) in innate and adaptive immunity. Inflammation is controlled by pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. NK cells use granzyme and perforin to destroy infected cells, B cells make antibodies (IgG in humans, IgY in chickens), and CD4 and CD8 T cells facilitate adaptive immunity.

In both species, immune control also incorporates checkpoints like PD-1 and CTLA-4.

Both require bone marrow for hematopoiesis and have a thymus for T cell maturation. B cell maturation is different in humans and chickens, taking place in the bursa of Fabricius for chicken and in bone marrow for humans. Both species immune responses are supported by secondary lymphoid organs such as the mucosa-associated lymphoid tissues (MALT), spleen, and lymphoid follicles. To defend against pathogens, both their immune cell types include granulocytes, dendritic cells, T and B lymphocytes, NK cells, and macrophages (M1/M2). Both species use innate and adaptive immunity, and key functions are played by phagocytosis, pattern recognition receptors (PRRs) like Toll-like receptors (TLRs), and cytokine signaling. Cytokines such as TNF-α, IL-6 and IL-1β control inflammatory reactions both in humans and chickens. While cytotoxic T cells ($CD8^+$) and T helper ($CD4^+$) mediate adaptive immunity and help in antigen-specific responses and immune memory, NK cells in both species eradicate infected cells via the perforin and granzyme pathways. B cells generate antibodies, known as IgG in humans and IgY in chickens, whereas T cell receptors (TCRs) use major histocompatibility complex (MHC) molecules to identify antigens. Furthermore, immunological checkpoints like CTLA-4 and PD-1 are used by both species to control immune development. [12] The purpose of this study is to assess the biotherapeutic product's therapeutic effectiveness and safety in wound healing. The study also looks at whether chickens may be used as a homologous animal model to evaluate regenerative wound healing treatments. By using this strategy, the study demonstrates the efficacy of the biotherapeutic product as well as the suitability of the chicken model for studies on wound healing.

2. Materials and Methods

2.1 Preparation and characterization of autologous therapeutic cells

To check the phenotypic and functional ability of the cells akin to those of human-derived cells, they were examined under a microscope. To verify their migratory capacity, which is crucial for tissue regeneration and wound healing, a scratch assay was conducted.

The wing vein was used to aseptically draw 3 mL of peripheral blood, which was then diluted 1:2 with sterile PBS and placed in tubes coated with EDTA. Density gradient centrifugation was used to separate peripheral blood mononuclear cells on HiSepTM LSM. After being collected and twice washed with DPBS, the mononuclear cell layer was resuspended in the suitable medium for therapeutic application. The Ficoll-based approach, as given by Bøyum, A. (1968), was followed throughout the procedure.[13] The cells were prepared following the same method used for preparing the human cells and characterized

for phenotypic and functional properties. The phenotypic characterization was done using the morphological properties of the cells. Functional migratory capacity was evaluated using an in vitro established scratch assay, in which cells were seeded in 12-well plates, allowed to reach 70–80% confluence, scratched using a sterile 1 mm pipette tip, and monitored for migration into the wound area. [14]

2.2 Study Design in the animal model

This study was conducted using four healthy 4–6-week-old broiler chickens. The birds received ad libitum and access to food and water during the study. A within-subject design was developed, where each chicken served as its own control to minimize inter-individual variability. Two full-thickness wounds (400 mm^2) were created on lateral thoracic region beneath the wings per bird. The left-side wound was treated with autologous cells and a right-side wound was left untreated and served as a control.

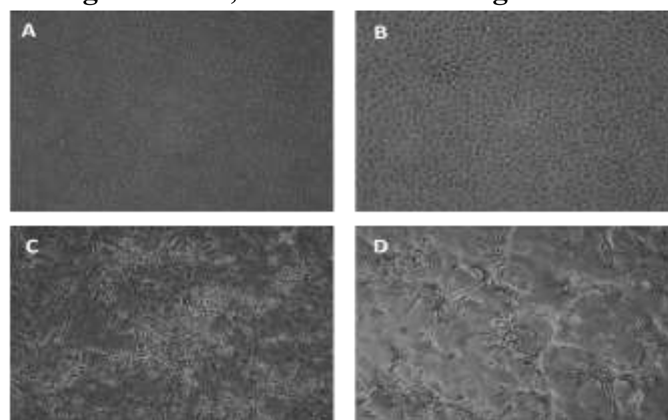
All procedures were performed under aseptic conditions using sterile surgical tools including scalpels, forceps, gauze, surgical scissors and all personnel adhered to sterile technique throughout the procedure. Lidocaine (2%) was administered locally prior to wound creation and the wounds were covered with sterile Tegaderm dressings. The subjects were monitored daily for signs of inflammation, infection, exudate formation and general health status to check the safety and efficacy of the product. Wound healing was observed for 10 days. Wound area was measured by determining the maximum length and width (mm) using a scale, and the area was calculated as length x width (mm^2). Percentage wound healing was calculated relative to the initial wound area. Data are presented as mean ± standard deviation (SD). Since each animal served as its own control, statistical comparison between treated and untreated wounds at each time point was performed using chi-square test, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Phenotypic characterization of the cells

Using the same protocol for generating human cells product, chicken peripheral blood-derived mononuclear cells were successfully isolated and cultured. Microscopic analysis showed that the cultivated cells exhibited spindle-shaped, fibroblast-like phenotypes and maintained a healthy morphology. As seen in Figure 1 (Panels A–D), cells maintained a similar morphology and adhesion across several days of in vitro treatment, indicating their stability and suitability for therapeutic usage.

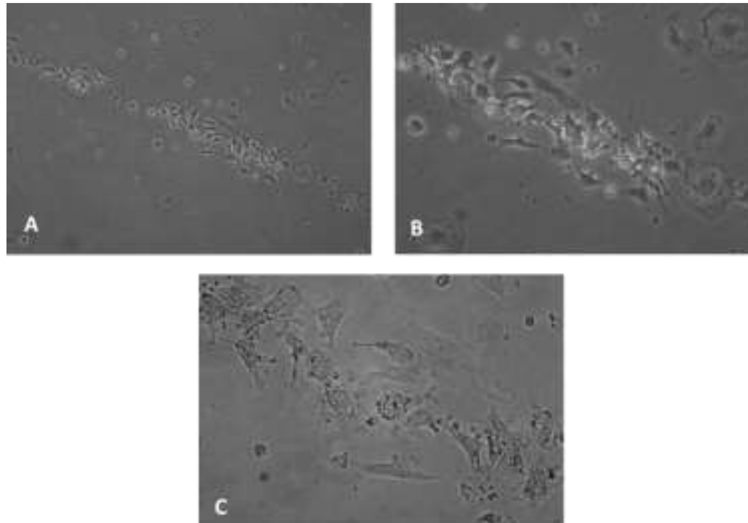
Figure 1: Therapeutic product derived from chicken using the same protocol used for generating human product, which showed similar phenotypic properties. Images A and C: 100x magnification; B and D: 200x magnification.



3.2 Functional characterization of cells using scratch assay

The modified chicken-derived therapeutic cells capacity to migrate was validated by the in vitro scratch assay. The scratched wound area gradually closed over the course of 24 to 48 hours, demonstrating active cell migration. A bridge monolayer formed over the scratch when examined under a microscope at various magnifications (100x, 200x, and 400x), indicating regeneration potential on par with results of human-derived cell therapy (Figure 2).

Figure 2. Therapeutic product derived from manipulated Chicken cells showed migratory potential similar to the human cells. A: 100x, B: 200x, C: 400x.



3.3 In Vivo Safety Assessment

During the 10-day follow-up period, macroscopic inspection of the wound sites showed no signs of systemic or local adverse effects in any of the treated birds. Figure 3 illustrates how treated wounds healed gradually and under control, showing no symptoms of bleeding, purulent discharge, severe inflammation, aberrant swelling, or tissue necrosis.

There was no discernible erythema extension past the wound borders, and the peri-wound skin seemed healthy. There were no indications of aberrant tissue development or abscess formation, and the scab formation was mild and well-organized. Crucially, after applying the cells, neither an excessive inflammatory response nor wound bed degradation was noted.

Figure 3. Representative images showing the healing of wound observed on different days following the treatment. The excision wound was created using a sterile scalpel and the wound size was measured prior treatment and on different interval following the treatment. In each bird, two wounds were created and one was treated and another was kept as untreated wound.

3.4 In Vivo Therapeutic Efficacy

When comparing autologous cell-treated wounds to untreated controls, macroscopic analysis showed considerably faster wound healing (Figure 3). Faster contraction, thinner scabs, better epithelialization, and better-organized wound borders were all seen in treated wounds. All of the birds getting therapy showed a similar progressive decrease in wound size. The visual observations were followed by quantitative assessments of wounds. On Day 10, 80–92% of treated wounds had closed, but only 41–65% of untreated wounds had closed as mentioned in Table 1. Compared to the untreated control group (56.75%), the treated group's mean percentage wound reduction was 87.25% greater where $p = 0.019$.

In comparison to controls (135–224 mm²), the final wound areas in treated locations were much reduced (minimum 35 mm²), suggesting improved contraction and tissue regeneration. A statistically significant decrease in wound area ($p < 0.05$) was also seen in the within-group comparison between Day 0 and Day 10, demonstrating successful healing process. In the chicken excision wound model, the autologous cells increase the wound contraction and facilitates effective tissue healing, as shown by the combined macroscopic observations and statistical analysis.

Table 1. Statistical difference in the wound area on before (day 0) and after (day 10) treatment.

Untreated Day0	Untreated Day10	P value	Treated wound Day0	Treated wound Day10	P value	Untreated %wound reduction	Treated %wound reduction	P value
378	135	0.004	418	35	0.000***	64	92	0.019*
380	224		462	54		41	88	
437	153		552	108		65	80	

360	154	420	45	57	89

*** These two groups are statistically significant at 95% confidence interval (the differences between the groups are significant).

4. Discussion

This study uses a homologous chicken excision wound model to assess the safety and therapeutic effectiveness of product It was possible to identify, cultivate, and characterize chicken peripheral blood-derived mononuclear cells using the same procedure that had been previously established for producing human therapeutic cells. During in vitro growth, the cells retained a consistent spindle-shaped, fibroblast-like morphology, demonstrating the protocol's cross-species adaptability and phenotypic stability. The scratch assay was used for functional characterization, which verified the cell's ability to migrate actively. The creation of a bridge monolayer and the gradual closure of the scratch region within 24 to 48 hours showed regenerative capacity, an essential characteristic needed for wound healing and re-epithelialization. These results imply that the cells biological action is maintained.

Further evidence of safety and effectiveness was provided by in vivo validation. The 10-day follow-up period revealed no clinical indications of systemic or local harm. Without severe inflammation, necrosis, purulent discharge, or aberrant immune responses, treated wounds healed under control. The lack of wound bed degradation and erythema extension promotes the autologous cells local tolerability and reduces major safety issues related to cell-based treatments. When compared to untreated controls, quantitative analysis showed that treated wounds had much more wound contraction. On Day 10, the mean closure rate for treated wounds was 87.25%, while the mean closure rate for untreated wounds was 56.75% ($p = 0.019$). Accelerated tissue regeneration is further supported by the reduced final wound areas in treated locations. The cells therapeutic value in this homologous model is reinforced by the statistical significance found.

Despite being extensively employed in studies on wound healing, rodent models have translational limitations because of variations in skin structure, immunological modulation, and wound healing processes. Although they have a greater physiological likeness to humans, large animal models like pigs and non-human primates come with complicated logistical issues, high upkeep expenses, and ethical issues. An affordable, easily available, and morally acceptable substitute for early-stage translational validation is the homologous chicken model.

But it's important to recognize the chicken model's limits as well. In particular, the lack of sweat glands and variations in dermal makeup distinguish avian skin from human skin in terms of structure. Wound healing dynamics may be influenced by feather follicles and weaker epidermal layers. Furthermore, compared to mammals, chickens naturally develop wounds more quickly, which may restrict the direct extrapolation of healing kinetics to humans. Even though they are conserved, several molecular pathways and cytokine responses may differ in the levels of expression or regulatory mechanisms between human and avian systems. Comprehensive mechanistic studies may also be limited by the lack of developed standardized immunological reagents and molecular tools for chickens in comparison to rodent models. Despite these drawbacks, chickens provide a physiologically relevant model for assessing the safety and initial effectiveness of autologous cell treatment due to their conserved immunological components, functioning T and B cells, cytokine signaling pathways, and similar tissue healing mechanisms. This study provides evidence that an autologous cell treatment approach may be effectively used to a

homologous chicken model, preserving therapeutic efficacy and safety while providing a useful intermediate platform for translational research.

5. Conclusion

In a homologous chicken excision wound model, this study shows that the autologous cell-based treatment is both safe and efficacious. The cells showed a great ability to migrate in vitro, maintained consistent phenotypic traits, and markedly accelerated wound healing in vivo without causing negative side effects. Statistically significant results demonstrated that treated wounds had much higher wound contraction (87.25%) than untreated controls (56.75%). The injectable autologous formulation's safety profile is further supported by the lack of systemic or local toxicity. For the preclinical assessment of cell-based regenerative therapeutics, the homologous chicken model offers an economical and physiologically appropriate platform. These results encourage this autologous treatment approach's further translational development toward clinical use.

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