The Critical Role of a Functional Golgi Apparatus in Optimal Wound Healing

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Section 004 BIOL 4620

December 8, 2023

Abstract

The Golgi apparatus is an extremely complex and important organelle. It plays essential roles in processing, sorting, and packaging of proteins and lipids. It has a close association with the cytoskeleton, which aids in cell movement and wound healing. If disrupted, the process of wound healing would be negatively impacted, having a detrimental effect to eukaryotes. Research is necessary in this area to understand the mechanisms behind the Golgi apparatus and the true part it plays in wound healing. The purpose of this experiment was to determine if the presence of a functional Golgi apparatus is critical for efficient wound healing. Brefeldin-A was used as an inhibitor due to its effects on the function of the Golgi apparatus. The control group was treated with normal growth media while the treatment group was treated with Brefeldin-A. Scratch 'wounds' were created on the monolayers and sizes were measured before and after a 20 hour incubation. The average difference in scratch size observed was significantly larger for the control than the treatment, meaning that the scratch wound decreased in size and was healed when the Golgi apparatus was not inhibited. This demonstrates that the Golgi apparatus is in fact essential for optimal wound healing, which supported our hypothesis.

Introduction

The Golgi apparatus is a vital cellular organelle that plays a crucial role in the complex process of wound healing. Situated within eukaryotic cells, the Golgi apparatus serves as a central location for the processing, sorting, and packaging of proteins, lipids, and other cellular materials. Its significance extends beyond routine cellular functions, as it actively participates in coordinating the cellular events essential for wound healing. Wound healing is a multifaceted biological response involving various cell types, signaling pathways, and structural elements. One key aspect of the Golgi apparatus's involvement in wound healing is its contribution to the synthesis and modification of proteins and lipids that are essential for cell migration, proliferation, and tissue repair. The Golgi apparatus processes these molecules and directs them to their specific destinations within the cell or for secretion, thereby influencing the cellular machinery that drives wound healing. Moreover, the Golgi apparatus is closely linked with the cytoskeleton, a dynamic network of protein filaments providing structural support and facilitating cellular movement. The cytoskeleton acts as an intricate scaffold during wound healing, guiding cell migration and maintaining the integrity of the cellular architecture. Interaction with the cytoskeleton is essential for the Golgi's role in wound healing.

Disruptions to the Golgi apparatus could have profound consequences on the mechanism of wound healing. If the Golgi apparatus is compromised, the processing and trafficking of crucial molecules would be severely impaired, leading to a breakdown in the coordination of cellular events during wound healing. The delivery of signaling molecules, growth factors, and structural components necessary for tissue regeneration could be disrupted, resulting in delayed or dysfunctional wound healing. Moreover, the Golgi apparatus's interaction with the cytoskeleton is integral to maintaining cellular structure and guiding migration, and any

disturbances in this interaction could further hinder the precise orchestration required for effective wound healing.

This calls into question the molecular mechanisms behind the Golgi's structure and function, and inspires research in this area so that we can further understand how the Golgi really contributes to keeping cells healthy. Eukaryotic cells adhering to surfaces exhibit the capability to undergo cell migration for wound repair, a phenomenon replicable in laboratory experiments. Given the dynamic nature of cytoskeletal filaments, manipulating cell behavior in terms of migration and monolayer formation is feasible by utilizing cytoskeletal inhibitors. In the controlled environment of a cell culture dish, a simulated wound, or "scratch," can be introduced into the monolayer. Subsequently, the cells are able to recuperate in the presence or absence of cytoskeletal inhibitors, and the healing outcome can be observed to assess their migratory and reparative abilities. There have been various approaches so far to assess the role of Golgi function. Scientists have created genetic mutations associated with the Golgi apparatus to observe its role in cellular functions. By using these mutations to disrupt the Golgi and its related proteins, the consequences of altered Golgi morphology and function can be studied. This can help show the functional significance of Golgi in processes such as protein trafficking and secretion, or even how these mutations lead to diseases and dysfunction. Another approach has been using chemical inhibitors that specifically target Golgi function. For example, scientists have used compounds like Brefeldin A and Monensin as Golgi disrupting agents due to their properties. By using these inhibitors, scientists can observe the effects on cellular processes that depend on the Golgi apparatus such as protein glycosylation and organelle organization. Therefore, our research question for this experiment was: To what extent does the functionality of the Golgi apparatus impact the efficiency of wound healing? Based on the background

information we knew and information gathered from sources, our hypothesis was that a functional Golgi apparatus is in fact crucial for optimal wound healing.

Materials and Methods

In this experiment, the hypothesis was tested based off of how much, quantitatively in μm, wound 'scratch' sizes shrunk based on their treatment groups. The control and experimental groups contained CHO cells not exposed to and exposed to the inhibitor, respectively. The cytoskeletal inhibitor used was Brefeldin A (BFA). BFA inhibits the function of the Golgi apparatus in mammalian eukaryotic cells. It functions by blocking the translocation of proteins between the endoplasmic reticulum and the Golgi apparatus. This drug can inhibit various mammalian cell lines at <40ng/ml. The results of the experiment were measured using microscopy capable of 100x magnification, both before and after a 20 hour incubation. The difference in the size of scratches before and after incubation was be used to analyze the results of treatment groups. The bigger the difference in scratch size before and after incubation, the more effective the wound healed.

The cell culture medium used was DMEM: F-12 supplemented with 1% (v/v) pen-strep and 5% NCS. Procedures were conducted underneath a Laminar flow hood cleaned with 70% ethanol. A 6-well plate was seeded with CHO cells grown to confluency. 20-200 μL pipettes with the tip on were used to perform the 'scratch' wounds. Single, parallel scratches were made perpendicular to the horizontal lines on each dish. Scratched monolayers were immediately imaged and width was measured. Growth medium was carefully removed from each dish by aspiration with a 5 mL pipette and discarded. 2 mL of the appropriate treatment solution replaced it in the first 3 wells and fresh growth medium without treatment were placed in the other 3 for

the control. Treatment solution was created with the correct dilutions of BFA from stock solution; 2 ng of BFA were put into 18 ng of stock, leading to a concentration of 0.1 ng/µL. Cells were then incubated at 37 deg celsius with 5% CO2 for 20 hours. Scatch widths were then remeasured using the same procedures as before. Statistical analysis was then performed using the data.

Results

	Width at 0 hours (µm)	Width at 20 hours (µm)	Difference (µm)
Control Well 1	629.5	453.7	175.8
Control Well 2	526.8	301.2	225.6
Control Well 3	533.2	352.8	180.4
Treatment Well 1	497.9	422.7	75.2
Treatment Well 2	496.8	432.6	64.2
Treatment Well 3	513.3	468.9	44.4

Figure 1. Data table showing the raw values of scratch width, in µm, obtained both before and after 20 hour incubation. Numbers were determined using a microscope capable of 100x magnification.

	Average Difference (µm)	Standard Deviation (µm)	Standard Error of the Mean (µm)
Control Group	193.93	27.52041666	15.89
Treatment Group	61.26	15.6081175	9.01

Figure 2. Data table showing the values of average difference, standard deviation, and standard

error of the mean, all in µm, based on the raw data obtained.

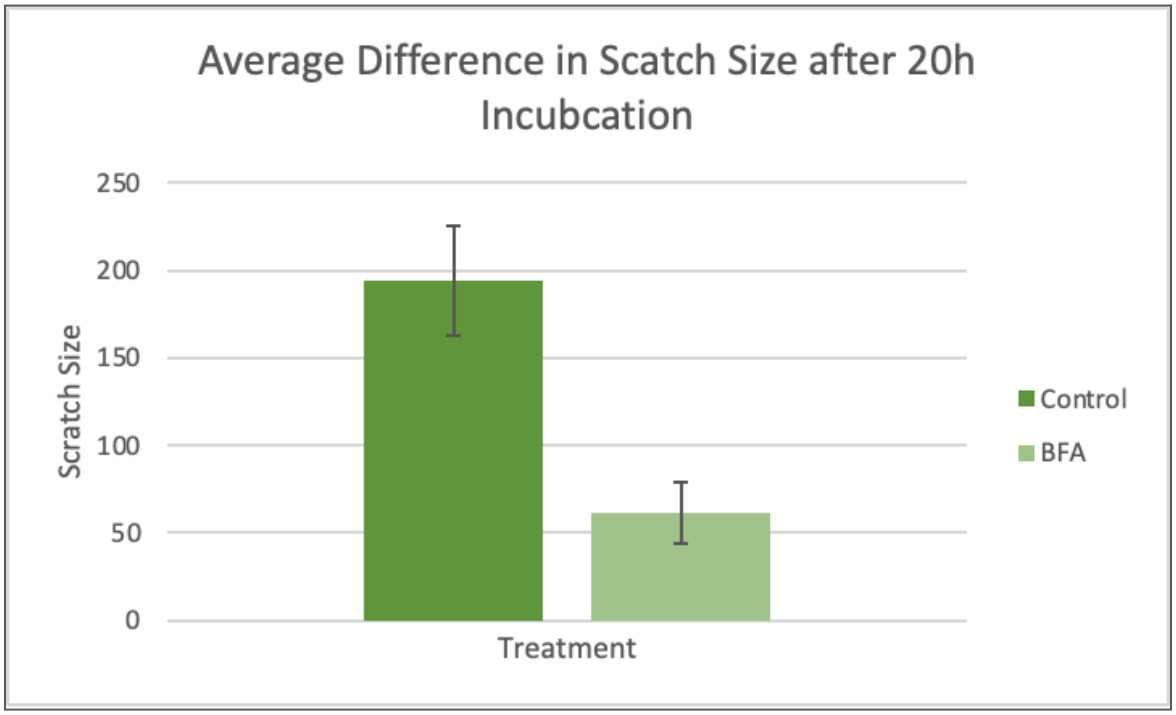

Figure 3. Bar graph demonstrating the average difference in scratch size, in µm, after a 20h incubation. Three wells were treated with normal growth media while three were treated with the inhibitor, BFA, and scratched carefully with a pipette tip. The p-value was found to be 0.00095 after running a t-test between the differences of control and treatment at various time points. The figure legend can be seen differentiating the control and BFA groups.

Treatments		p-values
Control 0hr	BFA Treatment 0hr	0.116 µm
Control 0hr	BFA Treatment 20hr	0.053 µm
Control 20hr	BFA Treatment 20hr	0.149 µm

Figure 4. T-test data demonstrating the significance of the results using p-values.

The results obtained show that the control had a higher average difference in scratch size,

measured before and after 20 hour incubation. The statistical analyses in Figure 2 demonstrate that the control also had a higher standard deviation and standard error of the mean in comparison to the BFA treatment. Figure 3 shows a visual representation demonstrating that the control had more of a difference in scratch width, at about 200 μm while the BFA treatment had a difference of about 50 μm. Figure 4 shows the p-values obtained after the t-test that prove that there is a significant difference between the average scratch size of the control versus the BFA treatment. All of these results were carefully observed and analyzed.

Discussion

Upon analyzing data from both of the treatment groups, it was observed that, after 20 hours of incubation, the average reduction in scratch size was significantly greater in the control group compared to the treatment group with BFA. In Figure 1, the raw data of measurements of scratch size before and after incubation can be seen. It can be deduced from this that the control wells with no treatment had more success in decreasing the scratch size, as the numbers are much higher than for the treatment. In Figure 2, statistical analyses including average, standard deviation, and standard error of the mean are shown for both the control group and treatment group. This also shows how the control group had higher values in each of these categories, demonstrating its success. In Figure 3, a bar graph can be seen in which the average scratch widths are visually displayed. The control bar is much higher than the treatment bar, and the standard error bars do not overlap. Specifically, the control exhibited an average reduction of approximately 200 μm, while the BFA-treated group showed a reduction of around 50 μm. This implies that the control group demonstrated more effective wound healing, as a higher difference signifies a greater decrease from the original scratch size. In Figure 4, p-values can be seen in

which comparisons of scratch size measurements at different time points between the control and treatment were made. The p-values indicate the statistical significance of the scratch wound sizes at these various time points. The healing process involves the Golgi apparatus reorienting itself towards the injury, crucial for maintaining cellular polarity and mobility throughout wound recovery. The Golgi-actin interaction is critical, allowing the Golgi to shift position, enabling Golgi dispersion and cell migration. The inhibitor BFA that was utilized in the experiment was found to impede anterograde transport by disassembling the Golgi apparatus. This interference with actin and microtubule assembly and disassembly hinders cellular mobility. The inhibitory role of BFA significantly impacted cellular function, resulting in suboptimal wound healing without any substantial decrease in scratch size after 20 hours. The experimental outcomes support our hypothesis that the Golgi apparatus plays an essential role in the wound healing process.

In a study conducted in 2022 by Purnati Khuntia, the Golgi apparatus was studied with wound healing regarding secondary epithelium. The experimental design was very similar to the one we used, and the results showed that when inhibited, wound healing did not occur nearly as effectively or efficiently as when the Golgi was working correctly. They also used the t-test to prove the significance of their results. They also found that microtubules and actin both proved to be very important for Golgi positioning. This supports and parallels the results that we obtained. In another study conducted in 2013 by Charbel Darido, a similar idea was focused upon; The functional necessity of Golgi structure and positioning for directed secretion and polarity in cell migration responses, such as wound healing. Again, very similar results were obtained. They state that when responding to a scratch wound, the establishment of cell polarity necessitates the Golgi apparatus's peri-centrosomal positioning. This suggests that, following

initiation by a polarity cue, there is a reliance on the Golgi's directed secretion to sustain the polarized state essential for facilitating cell migration and wound healing. Both of these studies further prove the necessity of the Golgi apparatus when it comes to wound healing.

While our study sheds light on the crucial role of the Golgi apparatus in wound healing, it is essential to acknowledge certain limitations that warrant consideration. Firstly, our investigation primarily focused on a specific cell type and may not fully capture the heterogeneity of cell responses in different tissue environments. Additionally, the study predominantly examined the Golgi apparatus's structural aspects, leaving the molecular and mechanistic details of specific signaling pathways involved in wound healing untouched. Moreover, the observed effects on wound healing were limited to in vitro settings, and translating these findings to actually in vivo or clinical scenarios requires cautious consideration. Furthermore, the study does not explore the potential influence of other cellular organelles or external factors that could contribute to the complex interplay during the wound healing process. Lastly, this experiment would definitely need to be repeated more to further verify the results.

With this being said, these limitations open avenues for future research with far-reaching implications. Understanding the intricate relationship between the Golgi apparatus and wound healing not only offers insights into fundamental cellular processes but also provides a basis for developing targeted therapeutic interventions and clinical applications in the future. Expanding the scope of investigation to include diverse cell types, tissue contexts, and in vivo models would enrich our understanding of the broader implications of Golgi dynamics in wound repair. Furthermore, delving into the specific molecular mechanisms and signaling pathways involved may unveil novel therapeutic targets for enhancing or accelerating the wound healing process. Overall, while our study provides a foundational understanding, addressing these limitations and

exploring the broader implications paves the way for a more comprehensive comprehension of the Golgi apparatus's significance in wound healing.

References

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