# How Do Vocal Folds Know When to Stop Healing?

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Medical research has its greatest value when it improves people's lives. As a postdoctoral researcher in the laboratory of Professor Susan L. Thibeault at the University of Wisconsin-Madison, I study how vocal folds repair after injury. My long-term goal is to help lay the groundwork for better treatments for people with voice disorders. I am honored to share my work through the Wisconsin Initiative for Science Literacy (WISL) program, Sharing UW-Madison Postdoctoral Scholarly Research with Non-Science Audiences. I sincerely thank the WISL team-Cayce Osborne, Elizabeth Reynolds, and Professor Bassam Shakhashiri-for their encouragement, thoughtful guidance, and commitment to making science accessible to everyone. Their efforts have helped me communicate my research.

For most of us, a voice feels effortless. We use it to work, teach, comfort, persuade, perform and connect with others. Yet we rarely think about the importance of our voice until it begins to fail.

**A Small Structure with a Big Job**

Our voice begins in the larynx, or voice box, where two small bands of tissue called the vocal folds stretch across the airway. When we speak, air from the lungs passes between them and causes their surfaces to vibrate, producing sound. Those vibrations can occur

hundreds of times every second (Jiang, Lin, & Hanson, 2000). To withstand that repeated motion, vocal folds need an unusual combination of properties. They must be strong enough to tolerate constant mechanical stress, yet soft and flexible enough to vibrate smoothly. Their layered structure makes this possible (Hirano, 1974). The outer surface is covered by a thin layer of cells called the epithelium, which forms a protective barrier. Beneath it lies soft connective tissue that allows the surface to move in a wave-like motion during speech. Deeper layers provide strength and elasticity, while muscle controls the position, shape, and tension of the vocal folds (Levendoski, Leydon, & Thibeault, 2014). Together, these layers turn airflow from the lungs into the sound we recognize as voice (Figure 1).

This sophisticated structure also creates a challenge when the tissue is injured. A vocal fold cannot simply close a wound. It must rebuild itself while preserving the delicate physical properties required for vibration.

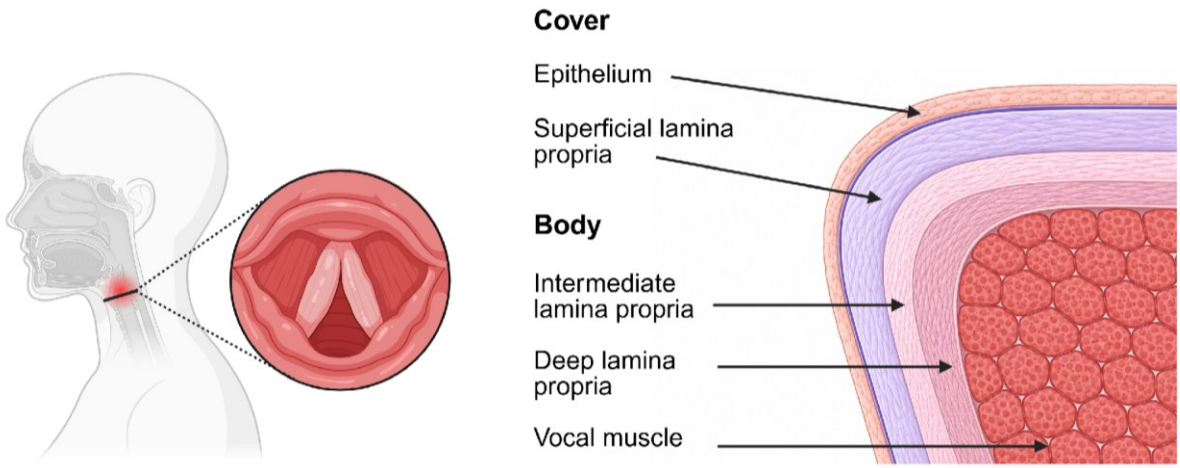



**Figure 1**. Schematic structure of the human vocal fold.

**Healing Requires an Accelerator and a Brake**

Vocal fold injuries can begin in many ways. Repeated mechanical stress can irritate the tissue, especially in people who use their voices intensely. Inflammation associated with

infection, smoking, environmental irritants, or reflux may also affect the vocal folds. Medical procedures involving the airway, including surgery and endotracheal intubation, can cause injury as well (Branski, Verdolini, Sandulache, Rosen, & Hebda, 2006).

When the vocal fold surface is damaged, the tissue responds quickly. The epithelium acts as the first protective barrier, so restoring that surface becomes an immediate priority (Leydon, Imaizumi, Yang, Thibeault, & Fried, 2014). Cells near the injured area become active. Some divide to replace cells that were lost. Others mature into the specialized cells needed to rebuild the lining. At first, the process sounds straightforward: damage occurs, cells multiply, and the wound closes (Bless & Welham, 2010). But successful healing requires much more coordination. The right cells must become active at the right time. They must divide in appropriate numbers. They must develop into the correct cell types and organize themselves into the proper layers. And then something equally important must happen: The repair response has to slow down. Imagine driving a car. You need an accelerator to get moving, but an accelerator without a brake would be dangerous.

Healing works in a similar way. A strong response is essential immediately after injury. But if cells continue dividing after enough tissue has been produced, the repaired surface may become too thick or develop an abnormal structure. More repair is not always better repair. This idea led me to the central question behind my research: What tells a vocal fold when enough repair is enough?

**The Quiet Cells Waiting for an Injury**

To answer that question, I became interested in a population of cells in the vocal fold epithelium that can be identified by a protein called *Lrig1*. These cells behave somewhat like a reserve workforce. When the tissue is healthy, many remain relatively quiet rather than

dividing continuously. But when injury creates a need for new cells, they can participate in rebuilding the epithelial surface. Previous work from our laboratory showed that a communication system called Notch1 signaling helps maintain this quiet state (Lungova, Fernandez, Cai, Kendziorski, & Thibeault, 2025). Notch signaling is one of the ways neighboring cells communicate with each other. It helps cells interpret their surroundings and make important decisions. One member of this system, a protein called Notch1, particularly interested me. If Notch1 helps keep *Lrig1*-expressing cells quiet in healthy tissue, what happens when an injury occurs and Notch1 is removed from those cells?

**How Notch1 Shapes Repair**

The laboratory mouse is a well-established model for investigating mechanisms of human disease and tissue repair. The mouse larynx shares key structural and cellular features with the human vocal folds. To investigate the role of Notch1 in vocal fold re-epithelialization after acute injury, I compared two groups of mice: mice with normal Notch1 activity and mice in which Notch1 was completely removed from *Lrig1*-expression cells. I activated this deletion by giving the mice tamoxifen, which acts as a genetic switch and triggers removal of Notch1 in those cells. This allowed me to compare healing when Notch1 was present with healing when it was absent from the targeted epithelial cells (Cai, Thibeault, & Lungova, 2026).

To create a controlled and reproducible epithelial injury, I used a chemical called naphthalene (NAPH), which researchers commonly use to damage the lining of the airway in a controlled way. I then examined the vocal folds at three stages of healing: one day, three days, and seven days after injury. First, I looked at the overall structure of the tissue under a microscope. A standard staining technique allowed me to see whether the epithelial

surface was intact, whether it had become unusually thick, and how its overall organization changed during recovery (Figure 2).

Second, I also used a technique called immunofluorescence. This method uses antibodies carrying fluorescent labels to identify particular proteins in tissue. Under a fluorescence microscope, those proteins appear as bright signals. I think of these signals as molecular signposts. Some tell me where cells are dividing. Others identify basal cells, a population of cells that sits along the bottom of the epithelium and can divide to help replace cells lost during injury. Still others reveal how epithelial cells are changing as they mature or how immune cells are accumulating in the injured tissue. These methods allowed me to follow not just whether the surface returned, but how the repair process unfolded over time.

**The Repair Response Goes Too Far**

One day after NAPH injury, the vocal fold surface was disrupted and thinner than in uninjured mice, with cellular debris visible around the damaged area (Figure 2). This acute damage was seen both in mice with normal Notch1 and in mice with Notch1 completely removed from *Lrig1*-expression cells. At this early stage, the most obvious effect was the injury itself; the effect of removing Notch1 became clearer as the tissue began to repair. By three days, the epithelium had become much thicker as cells multiplied to replace those that had been lost. This growth is a normal part of the repair process. However, the response was stronger in mice with deletion of Notch1. The layer of basal cells near the bottom of the epithelium expanded more, and more epithelial cells continued to divide than in mice with normal Notch1 (Figure 2).

The difference remained apparent one week after injury. In mice with normal Notch1, the repair response was beginning to settle. In mice without Notch1 in the *Lrig1*-expression

cells, however, the basal cells remained expanded and more cells continued to proliferate (Figure 2). The tissue was clearly able to respond to the injury, but it appeared to have more difficulty bringing that response back under control. These findings suggest that Notch1 does not simply turn repair on or off. Instead, it appears to help regulate the repair process, allowing the tissue to respond strongly enough to injury while also helping that response subside as healing progresses.

**When Healing Changes Course**

The previous findings showed that removing Notch1 prolonged the growth phase of repair. But successful healing requires more than producing enough new cells. Those cells also have to develop into the right types and organize themselves properly. After injury, epithelial cells showed changes associated with maturation and wound repair, and some of these differences remained one week later (Figure 2). This suggests that Notch1 helps guide not only how much the epithelium grows, but also how it rebuilds itself.

The effects also appeared to extend beyond the epithelial cells themselves. Healing involves the immune system, which helps clear damaged cells and support tissue repair. Three days after injury, these immune cells had accumulated in the injured tissue as expected during normal wound healing. However, more of these cells were present when Notch1 was completely removed, and this difference was still visible one week later.

This does not mean that Notch1 alone controls inflammation, the relationship is much more complicated than that. But my finding suggests that epithelial repair and inflammation may be closely connected—and that disrupting Notch1 in these epithelial cells can affect more than the epithelium itself.

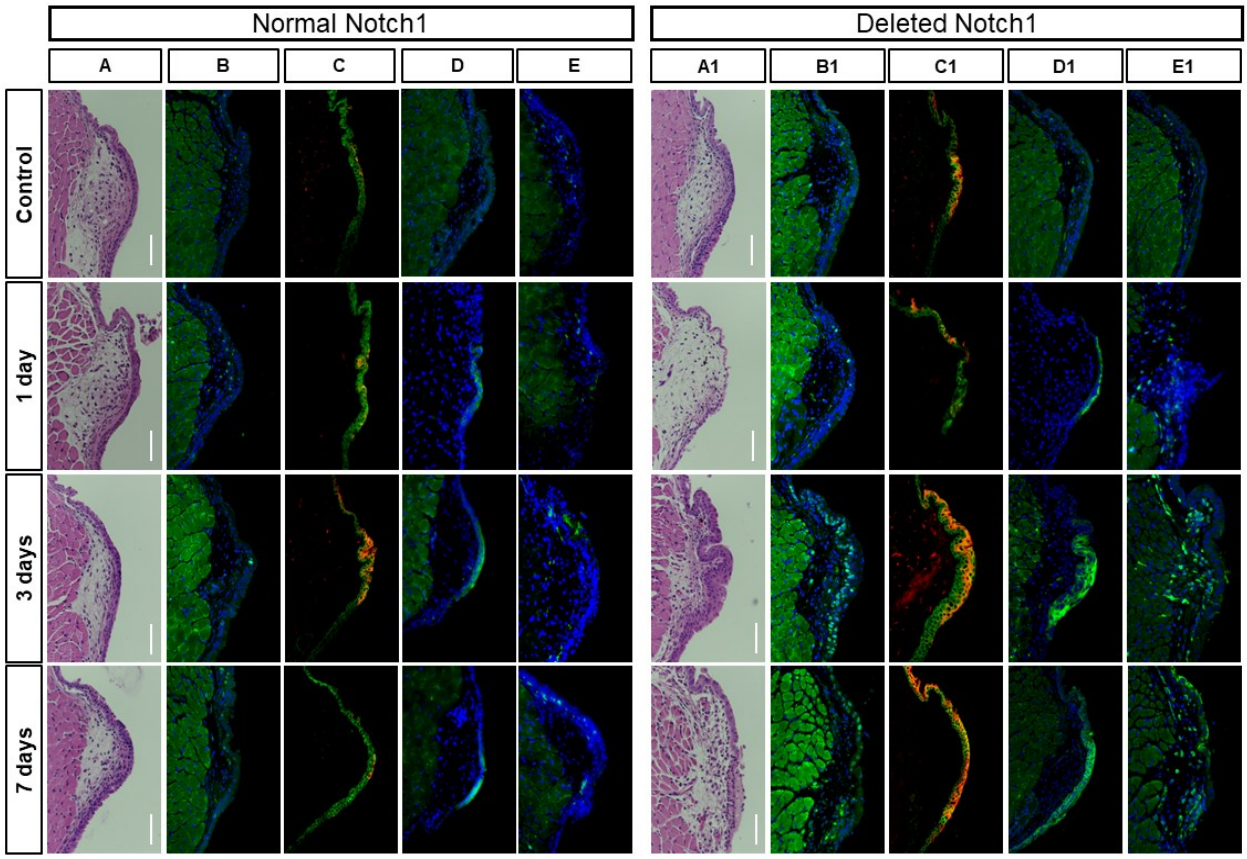



**Figure 2.** Vocal fold healing over time following injury in normal mice and with Notch1 deletion. Microscopic images show the vocal fold without NAPH injury (control) and at one, three, and seven days after NAPH injury. (A, A1) overall tissue structure. (B, B1) cells are actively dividing. (C, C1, D, D1) changes in epithelial maturation and injury response. (E, E1) the accumulation of immune cells . These images reveal how the vocal fold epithelium rebuilds itself and how the repair response changes over time when Notch1 is present or deleted.

**What the Research Taught Me**

When I began this study, I wanted to understand whether Notch1 influences repair of the vocal fold epithelium. The experiments led to a broader picture. When Notch1 was removed from *Lrig1*-expression cells in mice vocal folds, I observed three connected changes: Cells continued proliferating more strongly. The rebuilding epithelium showed altered patterns of

cell differentiation. And immune cell accumulation remained elevated for longer. Together, these findings suggest that Notch1 helps coordinate the timing and organization of vocal fold epithelial repair. That distinction matters. Healing is not simply a matter of making more cells as quickly as possible. A successful repair response must know when to begin, how strongly to respond, what kinds of cells to produce, how to organize them—and when to move from emergency repair back toward everyday maintenance. In that sense, healing is less like flipping a switch and more like conducting an orchestra. Cell proliferation, differentiation, tissue organization, and inflammation all have to occur at the appropriate time. If one part continues playing long after the others should have quieted, the final result may be very different.

**From Mice to Patients**

There is an important limitation to this research. My experiments were performed in mice. They cannot tell us exactly what happens in every person with a vocal fold injury, and they do not yet provide a new treatment for patients. Notch signaling also participates in many processes throughout the body. Understanding its role in an experimental model is very different from knowing whether—or how—it could ever be safely targeted as a treatment. Many questions remain. We still need to understand the molecular signals operating downstream of Notch1. We need to learn more about how epithelial cells communicate with immune cells during repair. And we need to determine how changes in the rebuilt epithelium ultimately affect the physical properties that are essential for vocal fold vibration. Those are not small steps.

But basic research provides the biological foundation on which future treatments are built. If we can understand how vocal fold cells decide when to divide, when to mature, and when to return to a resting state, we may eventually learn how to guide healing more precisely. The

goal would not simply be to make a wound disappear. It would be to help the vocal fold rebuild itself in the right way—restoring both tissue and function. For someone whose voice is central to a career, a relationship, artistic expression, or a sense of identity, that distinction could matter enormously.

When our voices work normally, speaking feels almost effortless. But beneath every sentence is an extraordinary biological performance. Cells sense damage. They communicate with their neighbors. Some begin dividing. Others change their identities. Immune cells enter the tissue. The protective surface is rebuilt. And then, somehow, the entire repair program has to quiet down at the right moment. All of this happens in a structure delicate enough to vibrate hundreds of times per second and strong enough to repeat that task day after day. My work on Notch1 is one small part of understanding how that balance is achieved. Each answer leads to another question. How does the epithelium communicate with the immune system while healing? What happens when the signals that normally coordinate those processes are disrupted? Those are the questions that continue to drive my research. Because recovering from a vocal fold injury should mean more than simply closing a wound. It should mean recovering the ability to speak, sing, teach, comfort, persuade, perform, and connect—the things that make a voice far more than just a sound.

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