THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 14 Issue 5

RESEARCH ARTICLE

Effects of Platelet-Rich Fibrin on Healing of the Vocal Fold in Rats

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OPEN ACCESS

PUBLISHED
31 May 2026

CITATION
Arslanoğlu, A., et al., 2026. Effects of Platelet-Rich Fibrin on Healing of the Vocal Fold in Rats. Medical Research Archives, [online] 14(5).

ISSN
2375-1924

ABSTRACT

Although scar formation following vocal fold surgery for benign diseases is rare, it has disastrous results. Platelet-rich fibrin has positive effects on wound healing and has been used in various disciplines for some time. This study aimed to evaluate the potential effects of platelet-rich fibrin on wound healing in the animal model's vocal folds.

Fourteen Wistar albino rats were included in the study. Six of them formed the fibrin-rich platelet group, and another six comprised the wound group. Two rats were monitored without any surgical intervention. Rats in the first group received fibrin-rich platelets after a surgical wound was created. The second group was observed without platelet-rich fibrin. Histopathological examination was performed on all vocal folds at the end of the second week. Inflammation in the epithelial layer and lamina propria, as well as vascular proliferation, was evaluated.

In the platelet-rich fibrin group, 33.4% of vocal folds showed inflammation, and 50% exhibited vascular proliferation. On the other hand, the second group had inflammation in 66.6% of rats and vascular proliferation in 83.3% of rats. The first group showed no inflammation in the lamina propria in 16.7% of cases, whereas the second group had inflammation in 100% of cases.

Platelet-rich fibrin may have some positive effects on vocal fold healing, but more comprehensive research is needed to confirm this.

Keywords: Platelet-rich fibrin, vocal fold, inflammation, acute damage, healing

Introduction

Scar formation is the leading cause of dysphonia following vocal fold surgery for benign diseases. The vocal fold is composed of squamous epithelium, superficial lamina propria, and deep lamina propria, which include collagen and elastin fibers. The extracellular matrix is made up of glycoproteins, proteoglycans, and glycosaminoglycans. These components determine the biomechanical properties of the vocal folds, and when scar tissue replaces them during wound healing, it disrupts their viscoelastic properties, resulting in the formation of surface waves. This disruption often leads to dysphonia due to impaired vibratory function, necessitating therapeutic interventions aimed at mitigating scar tissue development and preserving the vocal fold's intrinsic biomechanical characteristics. Various therapeutic modalities have been investigated to enhance vocal fold wound healing, including the application of hyaluronic acid and platelet-rich plasma, both of which have demonstrated beneficial effects on tissue regeneration and inflammation reduction[1].

Vocal fold scarring, which can result from surgical procedures, phonotrauma, or intubation, significantly damages the microstructure and elastic properties of the lamina propria, thereby hindering normal vocal fold oscillations. Such scarring may present as atrophy or sulcus formation within the vocal folds, worsening glottic insufficiency and reducing vocal quality.[3]

There have been many publications analyzing wound healing on the vocal folds and the materials that enhance it or reduce scar formation. Hepatocyte growth factor, echinacoside, mitomycin C, and mesenchymal stem cells have been reported to have positive effects. Platelet-rich fibrin (PRF) is composed of a high platelet volume in a low volume of serum. It is rich in thrombocytes and growth factors. Upon degranulation, these thrombocytes release a diverse array of growth factors, including transforming growth factor β, platelet-derived growth factor, and epidermal growth factor, which subsequently activate various cell types, inducing inflammation and facilitating wound healing.[5] The unique stratified architecture of the vocal fold lamina propria, particularly the superficial layer, renders it highly susceptible to scarring, as trauma at this level can stimulate excessive fibroblast activity and restrict the mobility of the integumentary layer.[6]

Research has reported that it enhances healing in soft, hard, and neural tissues. It contains growth factors including platelet-derived growth factor, transforming growth factor alpha, epidermal growth factor, fibroblast growth factor, and insulin-like growth factor. Moreover, it has been reported to support the immune system via leukotrienes, interleukin (IL), and leukocytes. However, its effects on wound healing in the vocal folds have not yet been studied. This study aimed to evaluate the effects of PRF on wound healing in the vocal folds. This investigation assessed the histological outcomes of PRF application in a rat model to elucidate its potential regenerative and anti-inflammatory properties in vocal fold tissue repair. Previous studies have characterized the dynamic cellular and genetic alterations that occur during vocal fold wound healing, noting that an initial inflammatory response, marked by fibroblast proliferation and intense extracellular matrix deposition, peaks between 3 and 8 days post-injury.[7] This rapid response in smaller mammals, while informative, highlights the need for careful extrapolation to human vocal fold healing, given the differences in biomechanical environment and structural complexity[10]. However, the specific mechanisms by which PRF influences the intricate process of vocal fold extracellular matrix remodeling and inflammatory modulation require further elucidation through targeted molecular and cellular analyses.

Material and Method

Approval was obtained from the Istanbul University Ethical Committee for the International Review Board, and the study was conducted at the Istanbul University Experimental Medicine Institute Laboratory. Four healthy Wistar albino rats, weighing between 300 and 3,50 grams, were used for the experiment. 4 cc of blood was collected from all rats to produce

PRF. Blood was collected in a tube without an anticoagulant (hematube) and placed into a Nuve NF200 centrifuge device (Ankara, Turkey). PRF was obtained after centrifugation at 400 g RCF for 9 minutes.

After rats were anesthetized with 50 milligrams per kilogram of ketamine hydrochloride (Ketalar®, Pfizer Warner Lambert, USA) and 5 milligrams per kilogram of xylazine hydrochloride (Rompun®, Bayer, Mefar İlaç Sanayi, Istanbul, Turkey), a nasal speculum and oropharyngeal retractor were used to expose the vocal folds. The rat's tongue was pulled forward, and the oropharyngeal lumen was dilated. Next, the vocal folds were visualized using a 0-degree 4 mm telescope (Karl Storz, HOPKINS Straight Forward, Germany). A microblade was utilized to incise the vocal folds. Six of the damaged vocal folds were treated with PRF, while the others were not. Two rats were spared from any surgical intervention to form the control group. The rats were followed for 11 days. Three of them experienced dyspnea on the first postoperative day but regressed spontaneously. They were maintained on a regular diet with free access to water and food. On the 11th postoperative day, they were euthanized with a high dose of Pentothal. Total laryngectomy was performed for pathological examination. Subsequently, the excised laryngeal specimens underwent fixation in 10% neutral buffered formalin for 24 hours, followed by decalcification in a formic acid solution to facilitate microtomy. Following decalcification, the tissues were processed through an ascending series of alcohol solutions, cleared in xylene, and embedded in paraffin blocks for sectioning. Sections of 5 µm thickness were then cut using a microtome and mounted on glass slides.

A single pathologist, blinded to the laryngeal specimens, examined them. After applying hematoxylin and eosin staining, the vocal folds were examined using a Nikon microscope (Eclipse E200, Japan). Inflammation in the vocal fold epithelium and lamina propria was evaluated separately. Epithelial inflammation was assessed as present or absent, while inflammation in the lamina propria was scored between 0-3 (0 - no inflammation; 1 - inflammatory cells constitute less than 20% of all cells under 200 magnification; 2 - inflammatory cells constitute between 20-50% of all cells; 3 - inflammatory cells constitute more than 50%). Vascularization was assessed as present or absent based on the number of vessels (more than four accepted as present under 200 magnification).

Results

There was no inflammation at the epithelium of the two control specimens. There was low-grade inflammation in the lamina propria in one area. No vascular proliferation was observed in this group. Examination of six vocal folds to which PRF was applied revealed epithelial inflammation in two cases, mild inflammation at the lamina propria in four cases, and moderate inflammation in one case. Vascular proliferation was noted in three cases (Table 1). The last group showed mild epithelial inflammation in four cases and three instances of lamina propria inflammation. Vascular proliferation increased in all cases, and the inflammatory infiltration was rich in lymphocytes.

The Chi-square test showed no statistical difference in epithelial inflammation ($p = 0.5,6,8$). Lamina propria inflammation also exhibited no difference ($p=0.3,11$). The difference in vascular proliferation was likewise insignificant ($p=0.1,2,7$).

Discussion

Healthy adduction and vibratory properties of the vocal folds should be maintained to produce a clear voice. If there is a problem with the epithelial surface or lamina propria, the vibratory pattern changes, leading to dysphonia. Polyps, cysts, nodules, and other benign pathologies disrupt vibration and prevent full adduction. Surgery may also cause scars that affect the phonation mechanism. The authors concluded that scar tissue formed, which disturbed voice quality. Despite the microflap technique and laser surgery, scar formation remains a significant issue. To understand the histopathological aspects of wound

healing and scar formation in the vocal cords, some studies were performed. They suggested that the macula flava could be the source of the extracellular matrix in the lamina propria and stated that cells in the macula flava have a chondroid nature and also produce a cellular layer in addition to the extracellular matrix. found that cellular proliferation occurred in the lamina propria, indicating that the macula flava did not provide a cellular layer. They proposed that the well-differentiated cells in the macula flava inhibited their proliferation.[12] This non-proliferative nature of macula flava cells suggests a more stable, less dynamic role in vocal fold remodeling than other fibroblasts, which readily induce hyaluronic acid production, which is crucial for tissue repair.[13] In contrast to these findings, other research has highlighted the dynamic capacity of fibroblasts within the vocal fold lamina propria to actively participate in extracellular matrix synthesis and remodeling, particularly in response to injury.[14] For instance, fibroblasts and macrophages are instrumental in hyaluronic acid production, a critical component for tissue hydrodynamics, cellular motility, and proliferation within the lamina propria, with its rapid turnover (3-5 day half-life) indicating its active role in tissue repair and potential fibrosis if dysregulated[15].

Wang et al. used fibroblast growth factor to prevent scar formation and concluded that it has the potential to prevent scarring. Similarly, they found that halofuginone altered collagen and elastin deposition following surgery. They analyzed the effects of PRF on rabbit vocal folds and demonstrated reduced granulation tissue and well-organized collagen deposition. They found that EGF, PDGF, TGF-Beta, and vascular endothelial growth factor levels were higher on the 11th day but showed no difference at the 12,16th week.[17]

They performed a prospective study on 4 patients. They injected collagen into the vocal cords to restore vibratory function. They followed the patients for 6-1, 8 months and reported improvements in vibration and adduction. On the other hand, acoustic analysis showed no improvement. Neuenschwander et al. applied autologous fat to the vocal folds of 7 patients to correct vibration. They achieved improvements in glottal closure and mucosal vibration, but the length of scar tissue did not change. Hsiung et al.[16] analyzed the results of autologous fat injections. They were evaluated through acoustic analysis and videolaryngostroboscopy (VLS). They detected increases in fundamental frequency and maximum phonation time. [18]

However, the efficacy of such interventions is often limited by the complex pathophysiology of vocal fold scarring, which involves alterations in extracellular matrix composition and viscoelastic properties, leading to persistent dysphonia[19,20]. Specifically, vocal fold scarring is characterized by disorganized and excessive collagen bundle deposition, diminished hyaluronic acid content, and altered expression of key extracellular matrix components, all contributing to increased tissue stiffness and reduced pliability. This pathological transformation hinders the restoration of normal mucosal wave propagation, thereby impacting phonatory function[22].

Jitter, shimmer, and the noise-to-harmonic ratio decreased. The Grade, Roughness, Breathiness, and Asthenicity scale showed improvements in grade, breathiness, and asthenicity. VLS indicated significant improvements in amplitude, mucosal wave, vibration, symmetry, and glottic closure. They applied hyaluronic acid and cow collagen and compared the two. Both groups showed improvement in glottic closure, but vibration, amplitude, and maximum phonation time were significantly better in the hyaluronic acid group. Additionally, absorption was less in the hyaluronic acid group. Given the challenges of completely restoring vocal fold function, novel strategies focused on regenerative medicine and tissue engineering, such as growth factor injections, are being explored to address tissue failure by promoting regeneration rather than merely repairing compromised tissue.[23] These approaches aim not only to reinforce mechanical properties but also to create a regenerative microenvironment, using biomaterials such as hyaluronic acid-derived hydrogels or calcium-based

hydrogels. Cell-based therapies, including the injection of multipotent regenerative cells, represent a powerful tool in regenerative medicine for laryngeal repair, promoting the production of extracellular matrix proteins in scarred or atrophied vocal folds.[2,8,28] For instance, tissue engineering approaches leverage scaffolds, regulatory signals, and various cell types to restore native extracellular matrix composition and biomechanical properties, which are often compromised in vocal fold scarring.[25,26]

Specifically, these strategies target the re-establishment of optimal viscoelasticity and pliability through the judicious application of biocompatible materials and cellular components to mitigate the effects of excessive collagen deposition and depleted hyaluronic acid[27,28]. Hyaluronic acid, a primary glycosaminoglycan in the vocal fold extracellular matrix, is a particularly promising biomaterial for such interventions due to its innate biocompatibility and critical role in regulating tissue viscoelasticity and pliability[29,30]. Its capacity to absorb and retain water contributes significantly to the tissue's shock-absorbing properties and ability to sustain vibrations[31]. Consequently, hyaluronic acid-based hydrogels are extensively investigated for vocal fold tissue engineering due to their non-inflammatory, non-immunogenic, and biocompatible characteristics, making them suitable for restoring the biomechanical integrity of the lamina propria[32,33].

This study aimed to assess the potential effects of PRF on vocal fold wound healing. We observed no significant improvements in healing, contrary to previous studies. Since these are the initial stages of wound healing and PRP is effective early on, we focused on these parameters. The main limitation of our study was the small sample size. Another weakness was the absence of functional and long-term results. Future research should include larger cohorts and longer follow-up periods to more thoroughly evaluate the long-term effectiveness and functional outcomes of PRF treatments in vocal fold wound healing.

Conclusion

PRF is a product derived from humans' own blood, so there is minimal risk of side effects. Although there were some differences, these were not significant. We think that the limited number of our cases could be a reason for this. Therefore, more comprehensive studies with larger numbers of cases and longer follow-up periods are required.

Acknowledgment:

This manuscript is based on the postgraduate thesis conducted by Ahmet Arslanoğlu.

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