# Design of a new fistula plug using acellular small intestinal submucosa with platelet-rich fibrin for anal fistula in rabbit model

**Nadia NR**
Research Center for Advanced Technologies in Cardiovascular Medicine, Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences

**Hasti Tashak Golroudbari**
Research Center for Advanced Technologies in Cardiovascular Medicine, Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences

**Armaghan Banikarimi**
Research Center for Advanced Technologies in Cardiovascular Medicine, Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences

**Asieh Heirani-Tabasi**
Research Center for Advanced Technologies in Cardiovascular Medicine, Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences

**Behnam Behboudi**
Colorectal Surgery Research Center, Imam Hospital Complex, Tehran University of Medical Sciences

**Mohammad Sadegh Fazeli**
Colorectal Surgery Research Center, Imam Hospital Complex, Tehran University of Medical Sciences

**Alireza Kazemeini**
Colorectal Surgery Research Center, Imam Hospital Complex, Tehran University of Medical Sciences

**Mohammad Reza Keramati**
Colorectal Surgery Research Center, Imam Hospital Complex, Tehran University of Medical Sciences

**Amir Keshvari**
Colorectal Surgery Research Center, Imam Hospital Complex, Tehran University of Medical Sciences

**Seyed Mohsen Ahmadi Tafti**
Smahmadit@sina.tums.ac.ir

Colorectal Surgery Research Center, Imam Hospital Complex, Tehran University of Medical Sciences

---

**Research Article**

**Keywords:** anal fistula, SIS, inflammation, fistula plug, tissue regeneration

**Posted Date:** April 5th, 2024

**DOI:** https://doi.org/10.21203/rs.3.rs-4201096/v1



**Additional Declarations:** No competing interests reported.

# Abstract

**Background:** Anal fistula is an abnormal tract between the anal canal and the perianal skin. Anal fistula plug (AFP) as a sphincter-preventing method has drawn more interest because of its simple procedure and low risk of incontinence. Small intestine submucosa (SIS) as a biomaterial plug has been applied in managing anal fistulae. Platelet-rich fibrin (PRF) is a platelet-derived product containing several growth factors. PRF has been widely used for soft tissue regeneration because it promotes angiogenesis and mitogenesis and inhibits inflammation in the wound site. Because of the rigid consistency of SIS and the low mechanical resistance of PRF and their tissue-regenerative properties, we created a novel SIS-PRF plug for managing anal fistula.

**Methods:** Anal fistulae were created in the rabbit model. 18 rabbits were used. Animals were divided into three groups, including SIS-PRF, SIS and control. Five weeks' post-treatment, animals were sacrificed, and fistula specimens were obtained.

**Results:** According to histological analysis, inflammation was significantly lower in the SIS-PRF group compared to other groups. Treatment with both biomaterials increased the number of closed fistula tracts, but it was markedly higher in the SIS-PRF group compared to the SIS group. No foreign body reaction was seen in the SIS-PRF group.

**Conclusions:** In this study, SIS-PRF reduced inflammation and increased connective tissue in fistulae. Taken together, SIS-PRF can be used in further studies about anal fistula management in humans.


# Introduction

An anal fistula is an abnormal tract between the anal canal and the perianal skin. It can be formed spontaneously or subsequent to an anal abscess and significantly impacts the quality of life through clinical manifestations such as inflammation, pain and purulent discharge (1). Fistula management aims to eliminate the fistula tract without sphincter dysfunction (2). Several procedures have been developed for the treatment of anal fistula including fistulotomy, cutting setons, advancement flaps and ligation of intersphincteric fistula tract procedure. Incontinence is the main drawback of these procedures with up to 35% rate of incontinence in advancement flaps and 36% in cutting setons(3, 4). Sphincter—preventing methods such as anal fistula plug (AFP) and glue were established to avoid this adverse effect. Fibrin glue is easily applicable and, in short-term follow-ups, showed promising results, but longer follow-ups showed poor and varying outcomes with success rates ranging from 14–85%(5, 6). The low success rates may be due to the inability of fibrin glue to completely fix within the fistula tract and lacking in-tissue-growth ability (5). AFPs have drawn more interest in these procedures because of their simple operation and low risk of incontinence (7). Different material has been used for this purpose, such as acellular dermal matrix (ADM), platelet-rich plasma (PRP) and SIS (2, 8, 9).

Over the last three decades, SIS as a biomaterial plug has been applied in managing anal fistulas (10). The absence of foreign body reaction, infection resistance, biodegradability, rigid consistency and

maintaining the host tissue properties make SIS an appropriate scaffold for fistula treatment. SIS can support cell migration and enable epithelial proliferation within the fistula tract. However, varying success rates (24–88%) and needing a long time for treatment are drawbacks of this biomaterial(11, 12).

PRF is a platelet-derived product containing several growth factors, notably vascular endothelial growth factor (VEGF), responsible for angiogenesis in the wound site. PRF has been widely used for soft tissue regeneration because it promotes angiogenesis and mitogenesis and inhibits inflammation in wound sites (13). Its anti-inflammatory effect has been linked to the migration of the host leukocytes to the fibrin network. It has also been used for the management of anal fistula, but the success rates were varying because of its insufficient consistency to support the soft tissue and low resistance to mechanical force (14, 15).

Because of the rigid consistency of SIS and the low mechanical resistance of PRF and its tissue-regenerative properties, we created a novel SIS-PRF plug for managing anal fistula. To the best of our knowledge, there is no evidence of using PRF and SIS simultaneously to manage anal fistulas. Therefore, in this study, we evaluated the efficacy of the SIS-PRF plug in managing anal fistulas.

## Materials and Methods

# SIS preparation

Bovine small intestines of 20–24 months old healthy male cows weighing 220 ± 15 kg were purchased from a veterinary-approved slaughterhouse and delivered to the laboratory in cold Phosphate Buffered Saline within the first hours of scarification. The tunica muscularis and tunica serosa layers of the intestine were removed mechanically from the submucosa of the small intestine and washed thoroughly with a normal saline solution. Afterwards, SIS sheets were placed in 1% Sodium Dodecyl Sulfate (SDS) and incubated in a shaker for 48h. Then, the SIS layers were rinsed with normal saline solution and incubated in Triton X-100, and afterwards, the detergent was removed with 1% pen/strep solution. Finally, samples were lyophilized for 8-10h using SCANVAC® Cool Safe.

# Decellularization assessment

For decellularization accuracy assessment, bSIS samples were fixed in 10% formalin solution, embedded in paraffin blocks, and sliced into 5µm sections. Each group contained four samples. Samples were stained with Hematoxylin and Eosin (H&E) and 40, 6-diamidino- 2-phenylindole (DAPI) stains to detect any residual nuclei in the acquired bSIS scaffold. Samples were then assessed by a fluorescence microscope (Olympus BX 53).

Also, DNA Quantification assays were performed. 100 mg of SIS powder was incubated with Protein K digestion buffer (100 mM NaCl, ten mM Tris-HCl (pH = 8), 25 mM EDTA (pH = 8), 0.5% SDS, 0.1 mg/mL proteinase K) at 50 ºC for 24h and then the digest was extracted using an alcoholic solution

(Isoamyl/phenol/chloroform alcohol 1:25:24) twice. Afterwards, ethanol and 3M sodium acetate were added to precipitate digested DNA.

Then, the DNA precipitation was centrifuged at 10000g for 10 min, and 1 ml of TE buffer (10 mM Tris and one mM EDTA) was added. QuantiT PicoGreen dsDNA Assay Kit (Invitrogen, USA) was recruited to calculate the DNA concentration in SIS samples following the manufacturer's instructions.

# SIS-PRF preparation

To prepare PRF, 10 ml of Rabbit blood was collected from the central vein of the rabbit ear and dispensed into two 5 ml plastic tubes and immediately centrifuged at 2700 RPM for 12 min. The middle layer of the centrifuged blood is almost depleted from Red Blood Cells (RBC) and consists of clotting factors that form a fibrin network trapping cytokine. Above this layer is a layer of acellularized plasma. The plasma layer was then collected and removed using a 3 ml pipette. The PRF middle layer was mechanically separated from the bottom layer using forceps and then was compressed to drain the entrapped fluid. Afterwards, the purified PRF was wrapped around the SIS scaffold to prepare the PRF-SIS plug for experimental application.

# Scanning electron microscopy (SEM)

SIS and SIS-PRF scaffolds were coated with gold for 180 seconds using a sputter coater (SC7620; Quorum Technologies, England) at the accelerating voltage set at 20 Kv and their Morphologic characteristics were evaluated by SEM (AIS2100; Seron Technology, South Korea).

# Biomechanical tests

Tensile strength of the SIS and SIS-PRF was measured using a Santam mechanical testing machine (STM-20, with a 200 N load cell, Iran). Six specimens from each group were used for testing. One-cm strips with 0.01 mm thickness and 5 mm width were used for the testing. For all measurements, 5 mm/min speed was applied. Ultimate Tensile strength, expressed in Newton and elongation-at-break of specimens were measured.

# Animals and Ethics

Eighteen male New Zealand white rabbits weighing 3–4 kg were purchased from the Pasteur Institute of Iran. All procedures in this study concord with the NIH guide for the care and use of laboratory animals. The study is approved by the Animals who had free access to rabbit standard chewing pallets and tap water ad libitum. Rabbits were housed in standard colony cages (1 rabbit per cage) at room temperature and with a light-to-dark cycle of 10h to 14h with proper air conditioning and 50% humidity. Two rabbits were randomly chosen for the pilot study, and the other 16 rabbits were randomly assigned into three groups of control (N: 4), SIS (N: 6), and SIS-PRF (N:6) for the main study

# Fistula creation

To create Fistula-In-Ano (FIA) model, all rabbits were put under general anaesthesia with an intramuscular injection of 10 mg. Kg-1 Xylazine and 30 mg. Kg-1 Ketamine prior to the procedures. Then, the perianal area of the animals was gently shaved using a razor blade, and 10% povidone-iodine solution was used to prepare the area. A spot was marked at the 9 O'clock position 2 cm apart from the lateral anal margin for the fistula tract creation. A 14G intravenous cannula was recruited to create the fistula tract from the external opening in the ischiorectal fossa to the internal opening on the dentate line. After fistula creation, the cannula introducer and the silicon elastomer sheath were removed from the tract. Ethibond 2 was passed through the tract and tied after silicon sheath removal to prevent fistula tract closure. Elizabeth's collars prevented animals from biting the ethibond and licking the anal fistula. These procedures were performed for all rabbits in the experimental groups. Rabbits were assessed every three days post-operation to monitor fistulae and to check

if the bands were in place. Twenty-six days post-operation, the fistula tracts and the peripheral granulation tissue resembled anal fistulae in humans, and the FIA model was established. Figure 1 shows a summary of what was done in this procedure.

# Fistula treatment

Rabbits were anaesthetized and prepared with the same method mentioned for fistula creation. Then, ethibonds were removed, and the fistula tract was identified with a fistula probe. For animals in the SIS group, SIS was twisted and gently inserted in the fistula tract using Maryland forceps pulling from the inner orifice to the outer orifice. Then SIS plug was fixed in the tract by single 5 – 0 polyglactin (Vicryl, NJ, USA) stitches in internal and external orifices. For the SIS-PRF group, SIS was prepared with the same method mentioned earlier and PRF was wrapped around the SIS bond to form the SIS-PRF plug. Then, the SIS-PRF plug was placed and fixed in the fistula tract with a similar method to the SIS plug, as shown in Fig. 2. The internal and external orifices were closed by 5 – 0 polyglactin (Vicryl, NJ, USA) stitches in all animals. Elizabeth collars were placed to prevent rabbit bites, and animals were macroscopically assessed every three days for five weeks post-treatment. After the follow-up period, rabbits were euthanized with an overdose of pentobarbital sodium (60 mg. Kg-1). The anorectal area was totally resected with the treatment plugs and put in 10% formalin solution for further histopathological assessments.

# Hematoxylin and Eosin (H&E)

Samples were embedded in paraffin blocks, cut into 5µm sections, and stained with Hematoxylin and Eosin. Specimens were assessed by a single pathologist blinded to the study using a light microscope (Olympus, CX43, MA, USA). Samples were graded on a scale from 0–3 (0: None, 1: Mild, 2: Moderate, 3: Severe) based on the severity of inflammation, neovascularization, fibroblast proliferation and White Blood Cells infiltration. Also, foreign body reaction, granulation tissue, and fistula tract were evaluated.

# Statistics

GraphPad Prism (ver 9.3.1; GraphPad Software, CA) was used for statistical analysis. Data were analyzed with Chi-square and Fisher's exact test. P values less than 0.05 were considered to be statistically significant.

# Results

### Fistula morphology

After five weeks, no visible fistula orifice was observed in any groups. The external appearance of the anus was mildly deformed in 2 rabbits in the control group, but all animals in SIS and SIS-PRF groups had a normal anal appearance. No discharge was seen at the site of the fistula or anus. No extrusion was seen during these five weeks in the SIS-PRF group.

### Scanning electron microscopy

SIS contained relatively straight fibers in a smooth background of tissue on the abluminal side and. It was denser and less fibrous on the stratum compactum side compared to the abluminal side. Abluminal side of the SIS-PRF scaffold showed the same as the abluminal side of the SIS one. But, on the stratum compactum side of the SIS-PRF scaffold, fine and even accumulations of cells (platelets and leukocytes) was seen (Figure3.A).

### Biomechanical tests

Tensile strengths showed no significant difference between two groups. Elongation-at-break also was similar between SIS and SIS-PRF groups (Table 1). Load-elongation curve of SIS as a sample can be seen in Figure 4.

### Table 1. Biomechanical tests

| Groups | SIS (n=6) | SIS-PRF (n=6) | P-value |
|---|---|---|---|
| Tensile strength (N) | 4.49 ± 0.17 | 4.51 ± 0.21 | 0.89 |
| Elongation-at-break (mm) | 1.64 ± 0.10 | 1.63 ± 0.13 | 0.94 |

### Histopathological analysis

Although macroscopic investigation showed only two open fistula tracts in the control group, microscopically, there were 4 and 1 remaining fistula tract in the control and SIS groups, respectively. In the control group, moderate and severe inflammation was seen in the site of the fistula tract (figure 5. C). Inflammation was reduced in the SIS group, but the SIS-PRF group was significantly less inflamed than the SIS group (p-value = 0.01). Two rabbits in the control group had severe inflammation with concurrent macroscopic open fistula tract while animals in other groups did not show severity in histological analysis. All the animals in the SIS-PRF group showed mild inflammation. In the SIS group, mild

inflammation was seen in 2 animals, and others showed moderate inflammation. The only microscopic open fistula tract in the SIS group had moderate inflammation. There was also a significant decrease in the number of microscopically open fistula tracts in the SIS-PRF group compared to the control group.

A mild foreign body reaction was seen in one of the rabbits in the SIS group, but it was not significantly comparable to other groups. Granulation tissue was observed in one of the animals in the control group, which was one of the rabbits with a macroscopic open fistula tract.

We also compared groups according to neovascularization, but there was no difference between groups.

Connective tissue formation was comparably higher in the SIS-PRF group than SIS (p-value =0.55) and control (p-value=0.22) groups. A brief summary of the histological analysis is shown in Table 2.

**Table 2. Histological analysis**

| Groups | control | P1-value | SIS | P2-value | PRF-coated SIS | P3-value |
|---|---|---|---|---|---|---|
| Fistula tract (open or closed)[1] | 0.6 (0-1) | 0.09 | 0.16 (0-1) | 0.34 | 0 (0-1) | 0.01 |
| Neovascularization[2] | 1 (1-3) | NA | 1 (1-3) | NA | 1 (1-3) | NA |
| Inflammation severity[2] | 2 (1-3) | 0.49 | 1.6 (1-3) | 0.01 | 1 (1-3) | <0.001 |
| Foreign body reaction[1] | 0 (0-1) | 0.34 | 0.16 (0-1) | 0.34 | 0 (0-1) | NA |
| Granulation tissue formation[1] | 0.16 (0-1) | 0.34 | 0 (0-1) | NA | 0 (0-1) | 0.34 |
| Connective tissue formation[2] | 1.3 (1-3) | 0.42 | 1.6 (1-3) | 0.55 | 1.8 (1-3) | 0.22 |

P1-values are results of the comparison of control and SIS groups. P2-values are results of the comparison of SIS and PRF-coated SIS groups and P3-values are results of comparison of control and PRF-coated SIS groups

Chi-square test was used for statistical analysis

[1] Values are shown as -=0 and +=1

[2] Values are shown as 1=mild, 2=moderate and 3=severe

# Discussion

This study shows that the SIS-PRF plug is an effective treatment for managing anal fistula. A lower amount of inflammation and a higher amount of connective and hair follicle formation were seen in the SIS-PRF group compared to the other groups.

Rabbits are used extensively for establishing anal fistula due to their resemblance to human's anorectal anatomy and suitable body size (16, 17). Among different timings used for fistula creation, we chose the timing introduced in a study by Qin et al. in which the central fistula was similar to the clinical features of anal fistula 26 days after fistula creation (17).

As a novel therapeutic approach, tissue engineering is effective in different gastrointestinal diseases, such as oesophagal defects and anal fistulae (18). In this field, scaffolds can enhance wound healing (19).

As an autologous blood product, PRF is a proper scaffold for cell migration and can supply a slow release of growth factors and cytokines at the application site (20, 21). Besides these properties, soft tissue healing and anti-inflammatory characteristics of PRF have made it a suitable material to fill cavities in plastic surgery (22, 23). Platelet-derived growth factor (PDGF) released by PRF is attributed to its anti-inflammatory property. Using PRF as both a clot and a membrane has been shown effective in the oroantral fistula (24). In a study by Chuang et al. It was shown that adding PRF to adipose tissue–derived stem cells (ADSC) can increase ADSC's therapeutic effect on rat sciatic nerve injury. Studies evaluating the impact of PRF on anal fistula have resulted in a recurrence rate varying from 10–34%(14, 25). PRF lacks the solid state needed for fistula management and can't be fixed entirely within the fistula tract (26). Therefore, it is easily extruded from the fistula and may explain the high recurrence rate.

SIS is a commonly used scaffold that allows fibroblast migration and accelerates tissue healing (27). Its good cell adhesion, mechanical property, and being easily formed into a plug make it a proper fistula plug that cannot be easily extruded from the tract(26). It was shown that fistulae treated with SIS were more similar to the normal anal structure than the control group, which received PBS(12). The use of SIS in different clinical studies, such as an abdominal wall or bladder defect and anal fistula, has shown promising results due to increased proliferation and cell migration in the site of the fistula tract(28–30). In a study by Franklin et al., 53 patients underwent laparoscopic repair using SIS. After a 2-year follow-up, no recurrence was observed among patients(31). In our study, SIS-treated fistulae were less inflamed and better healed than the control group, but SIS-PRF showed better results than the SIS group, demonstrating the possible additive effects of PRF on SIS. Considering that the only two macroscopic open fistula tracts were the only ones with severe inflammation, it might be concluded that fewer open fistula tracts in the SIS-PRF group result from the lower amount of inflammation. Also, no foreign body reaction was observed in the SIS-PRF group, which can result from PRF being an autologous product. Despite the angiogenic properties of PRF noted in previous studies, no significant difference was observed in the SIS-PRF group due to the low amount of PRF covering SIS or the relatively short healing time.

In our study, SEM showed that besides some cellular aggregations in SIS-PRF scaffolds, no other differences were noticeable between the two groups. These cell aggregations have been proved to be

platelets and leukocytes that play important roles in releasing growth hormones and improving wound healing (32). According to the mechanical test results, SIS-PRF showed a similar strength compared to the SIS scaffold. This means that adding PRF to SIS as a collagen-rich scaffold does not affect its integrity and firmness. Tensile strength of SIS makes it a proper option for soft tissue repair such as abdominal wall repair (33–34).

There are some limitations in our study. First, lacking a solid-state made it impossible to compare the PRF separately with other groups. Therefore, whether the PRF is more effective than SIS in SIS-PRF is unknown. We also couldn't find notable granulation tissue in our histologic evaluation in any groups, possibly due to the relatively short time of fistula creation. Another study with longer fistula creation time may lead to its formation. More extensive studies concerning cytokine and growth factor formation can clarify the role of PRF or SIS in the healing process of anal fistula.

# Conclusions

In this study, SIS-PRF reduced inflammation and increased connective tissue in fistulae. Taken together, SIS-PRF can be used in further studies about anal fistula management in humans.

# Abbreviations

**ADM:** acellular dermal matrix

**AFP:** anal fistula plug

**DAPI:** 40, 6-diamidino- 2-phenylindole

**FIA:** fistula-In-Ano

**SIS:** small intestine submucosa

**H&E:** Hematoxylin and Eosin

**PRF:** platelet-rich fibrin

**PRP:** platelet-rich plasma

**RBC:** Red Blood Cells

**VEGF:** vascular endothelial growth factor

# Declarations

### Authors contribution

NR and SMAT contributed to the study conception and design. Material preparation, data collection and analysis were performed by HTG, NR, AB, BB, AK, AK, AHT, MRK and MSF. The first draft of the manuscript was written by NR and HTG and AB. All the authors read and approved the final manuscript.


### Acknowledgments

None.

### Funding

None.


### Declaration of conflicting interests

The Authors declare that there is no conflict of interest.

### Ethics approval

Ethics committee of Tehran University of Medical Sciences approved the present study. It was performed in accordance to the 1964 Declaration of Helinski.

### Data availability

The data set analyzed during the present study are available from the corresponding author on reasonable request.

## References


1. Sugrue J, Nordenstam J, Abcarian H, Bartholomew A, Schwartz JL, Mellgren A, et al. Pathogenesis and persistence of cryptoglandular anal fistula: a systematic review. Tech Coloproctol. 2017;21(6):425-32.
2. Han JG, Xu HM, Song WL, Jin ML, Gao JS, Wang ZJ, et al. Histologic Analysis of Acellular Dermal Matrix in the Treatment of Anal Fistula in an Animal Model. Journal of the American College of Surgeons. 2009;208(6):1099-106.
3. Hammond T, Knowles C, Porrett T, Lunniss P. The Snug Seton: short and medium term results of slow fistulotomy for idiopathic anal fistulae. Colorectal Disease. 2006;8(4):328-37.
4. Shawki S, Wexner SD. Idiopathic fistula-in-ano. World journal of gastroenterology: WJG. 2011;17(28):3277.
5. Sentovich SM. Fibrin glue for anal fistulas: long-term results. Diseases of the colon & rectum. 2003;46:498-502.
6. Loungnarath R, Dietz DW, Mutch MG, Birnbaum EH, Kodner IJ, Fleshman JW. Fibrin glue treatment of complex anal fistulas has low success rate. Diseases of the colon & rectum. 2004;47:432-6.

7. Zhao M, Wang A, Zhang L, Yu H. Establishment of a novel experimental model of infected anal fistula in rat. Laboratory Animal Research. 2022;38(1):15.
8. Champagne BJ, O'Connor LM, Ferguson M, Orangio GR, Schertzer ME, Armstrong DN. Efficacy of anal fistula plug in closure of cryptoglandular fistulas: long-term follow-up. Diseases of the colon & rectum. 2006;49:1817-21.
9. Moreno-Serrano A, García-Díaz JJ, Ferrer-Márquez M, Alarcón-Rodríguez R, Álvarez-García A, Reina-Duarte Á. Using autologous platelet-rich plasma for the treatment of complex fistulas. Rev Esp Enferm Dig. 2016;108(3):123-8.
10. Lewis R, Lunniss P, Hammond T. Novel biological strategies in the management of anal fistula. Colorectal Disease. 2012;14(12):1445-55.
11. Cintron JR, Abcarian H, Chaudhry V, Singer M, Hunt S, Birnbaum E, et al. Treatment of fistula-in-ano using a porcine small intestinal submucosa anal fistula plug. Tech Coloproctol. 2013;17(2):187-91.
12. Qin X, Wang P, Huang Y, Li Y, Chao M, Wang W. Adipose-derived stem cells are an efficient treatment for Fistula-in-ano of Japanese rabbit. Stem Cells International. 2019;2019.
13. Miron R, Choukroun J. Use of Platelet Rich Fibrin in Other Areas of Medicine. Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications. 2017:237-49.
14. Lara FJP, Serrano AM, Moreno JU, Carmona JH, Marquez MF, Pérez LR, et al. Platelet-Rich Fibrin Sealant as a Treatment for Complex Perianal Fistulas: A Multicentre Study. Journal of Gastrointestinal Surgery. 2015;19(2):360-8.
15. Pérez Lara FJ, Hernández González JM, Prieto-Puga Arjona T, Moya Donoso FJ, Doblas Fernández J. A new, conservative treatment for perianal fistula that may halve the need for surgical intervention: case series. Surgical Innovation. 2022;29(1):50-5.
16. Benlice C, Yildiz M, Baghaki S, Erguner I, Olgun DC, Batur S, et al. Fistula tract curettage and the use of biological dermal plugs improve high transsphincteric fistula healing in an animal model. International journal of colorectal disease. 2016;31(2):291-9.
17. Qin X, Wang P, Huang Y, Li Y, Chao M, Wang W. Adipose-Derived Stem Cells Are an Efficient Treatment for Fistula-in-ano of Japanese Rabbit. Stem Cells Int. 2019;2019:6918090.
18. Lysaght MJ, Reyes J. The growth of tissue engineering. Tissue engineering. 2001;7(5):485-93.
19. Nazari H, Naei VY, Tabasi AH, Badripour A, Akbari Asbagh R, Keramati MR, et al. Advanced Regenerative Medicine Strategies for Treatment of Perianal Fistula in Crohn's Disease. Inflammatory Bowel Diseases. 2021;28(1):133-42.
20. Garin P, Mullier F, Gheldof D, Dogne J-M, Putz L, Van Damme J-P. Platelet-rich fibrin (PRF): an autologous packing material for middle ear microsurgery. B-ENT. 2014;10(01):27-34.
21. Strauss F-J, Nasirzade J, Kargarpoor Z, Stähli A, Gruber R. Effect of platelet-rich fibrin on cell proliferation, migration, differentiation, inflammation, and osteoclastogenesis: a systematic review of in vitro studies. Clinical oral investigations. 2020;24:569-84.

22. M. Dohan Ehrenfest D, Bielecki T, Jimbo R, Barbe G, Del Corso M, Inchingolo F, et al. Do the Fibrin Architecture and Leukocyte Content Influence the Growth Factor Release of Platelet Concentrates? An Evidence-based Answer Comparing a Pure Platelet-Rich Plasma (P-PRP) Gel and a Leukocyte- and Platelet-Rich Fibrin (L-PRF). Current Pharmaceutical Biotechnology. 2012;13(7):1145-52.
23. Inchingolo F, Tatullo M, Marrelli M, Inchingolo AM, Scacco S, Inchingolo AD, et al. Trial with Platelet-Rich Fibrin and Bio-Oss used as grafting materials in the treatment of the severe maxillar bone atrophy: clinical and radiological evaluations. European review for medical and pharmacological sciences. 2010;14 12:1075-84.
24. Parvini P, Obreja K, Sader R, Becker J, Schwarz F, Salti L. Surgical options in oroantral fistula management: a narrative review. International Journal of Implant Dentistry. 2018;4(1):40.
25. Ramachandran R, Gunasekharan V, Pillai AV, Raja S, Nair AS. Fibrin glue versus autologous platelet-rich fibrin - comparison of effectiveness on the cohort of patients with fistula-in-ano undergoing video-assisted anal fistula treatment. J Minim Access Surg. 2022;18(3):443-9.
26. Wang T, Liu S, Wang W, Shu Z, Luan Z, Huang Y, et al. Injectable and Self-healing microgels repairing infected irregular tubular fistula in anal and muscle. Chemical Engineering Journal. 2022;441:135948.
27. Johnson EK, Gaw JU, Armstrong DN. Efficacy of anal fistula plug vs. fibrin glue in closure of anorectal fistulas. Diseases of the colon & rectum. 2006;49:371-6.
28. Cintron J, Abcarian H, Chaudhry V, Singer M, Hunt S, Birnbaum E, et al. Treatment of fistula-in-ano using a porcine small intestinal submucosa anal fistula plug. Techniques in coloproctology. 2013;17:187-91.
29. Cheng EY, Kropp BP. Urologic tissue engineering with small-intestinal submucosa: potential clinical applications. World journal of urology. 2000;18:26-30.
30. Ueno T, Pickett LC, de la Fuente SG, Lawson DC, Pappas TN. Clinical application of porcine small intestinal submucosa in the management of infected or potentially contaminated abdominal defects. Journal of gastrointestinal surgery. 2004;8:109-12.
31. Franklin ME, Gonzalez JJ, Glass JL. Use of porcine small intestinal submucosa as a prosthetic device for laparoscopic repair of hernias in contaminated fields: 2-year follow-up. Hernia. 2004;8(3):186-9.
32. Crisci A, Manfredi S, Crisci M. Fibrin rich in Leukocyte-Platelets (L-PRF) and Injectable Fibrin Rich Platelets (I-PRF), two opportunity in regenerative surgery: Review of the sciences and literature. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2019;18(4):66-79.
33. Ramaraju H, Ul-Haque A, Verga AS, Bocks ML, Hollister SJ. Modulating nonlinear elastic behavior of biodegradable shape memory elastomer and small intestinal submucosa (SIS) composites for soft tissue repair. Journal of the mechanical behavior of biomedical materials. 2020;110:103965.
34. Cao G, Wang C, Fan Y, Li X. Biomimetic SIS-based biocomposites with improved biodegradability, antibacterial activity and angiogenesis for abdominal wall repair. Materials Science and Engineering: C. 2020;109:110538.

# Figures

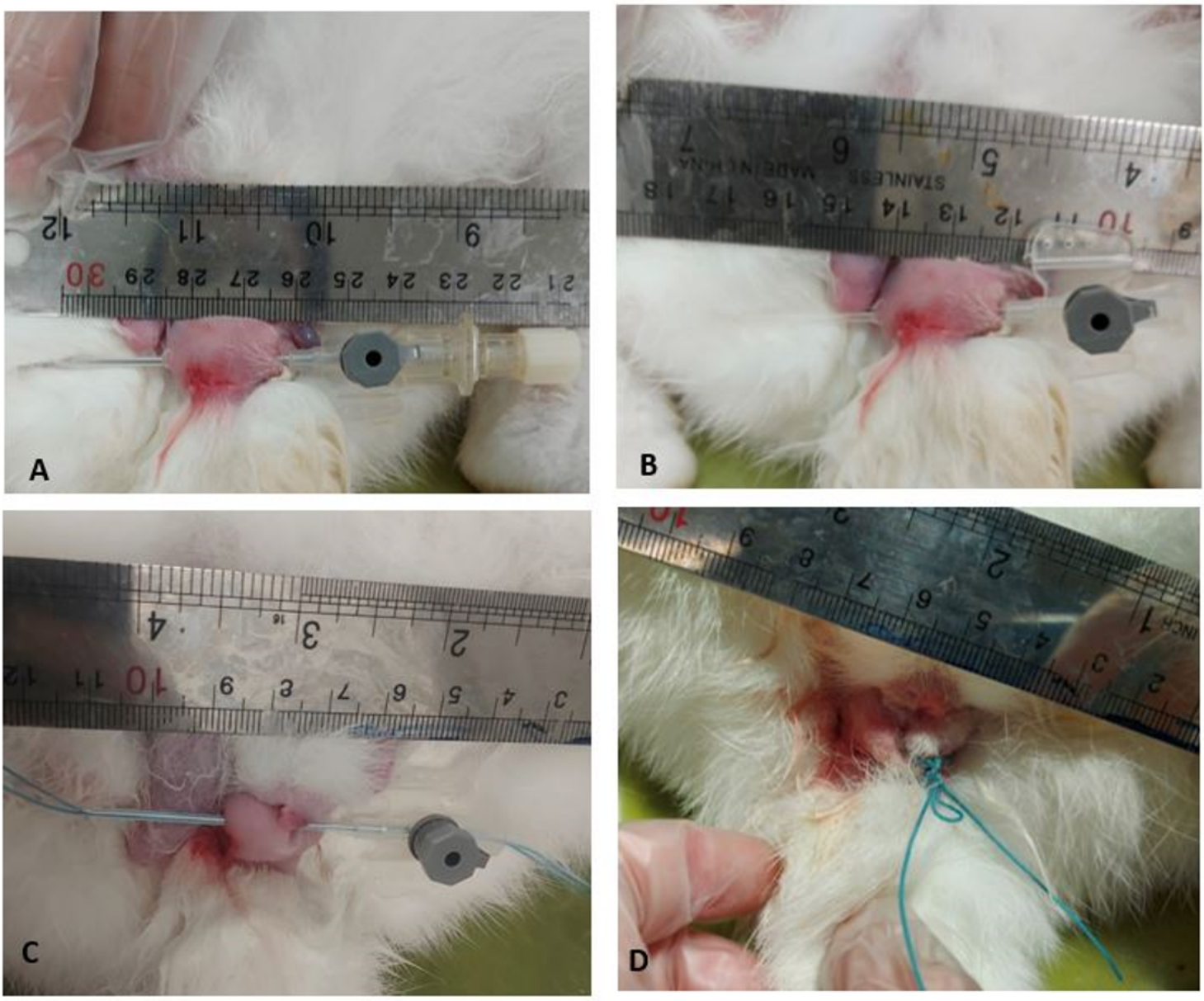
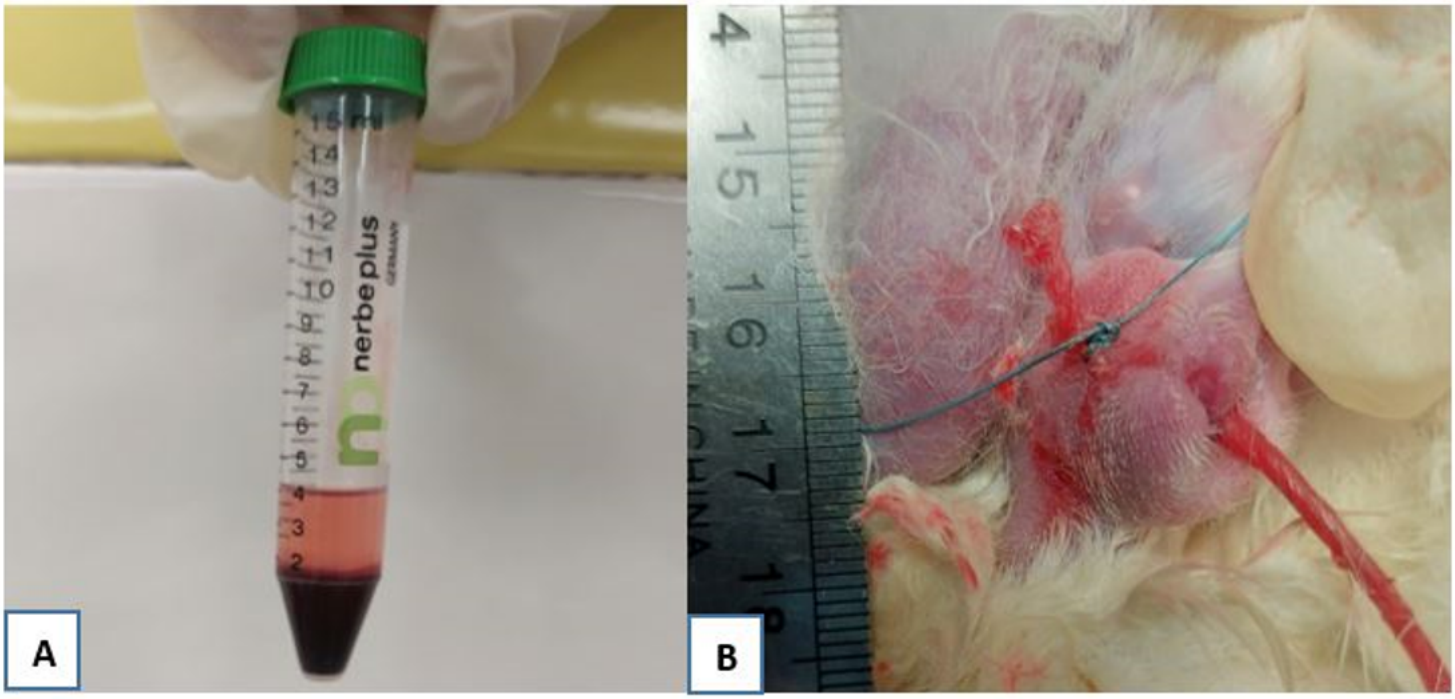


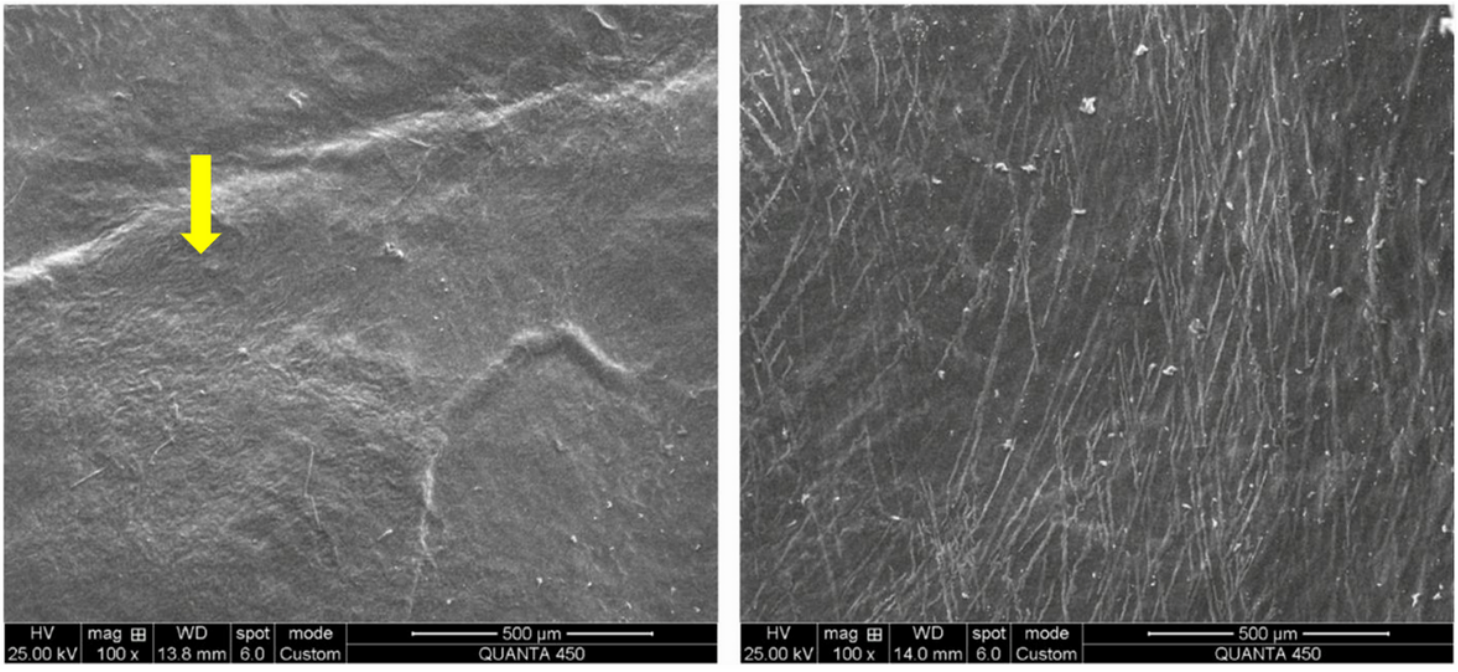

**Figure 1**

Fistula creation. **A** An intravenous cannula was inserted at 9 o'clock position 2 cm left to the anal orifice. **B** Needle was removed and the sheath was remained in the position. **C** Ethibond was passed twice through the sheath. **D** The sheath was removed and the Ethibond was tied in the created tract.




## Figure 2

**A**PRF formed in middle layer of the tube. **B** fixation of PRF-coated SIS in fistula tract.




## Figure 3

A shows the stratum compactum view of the SIS-PRF scaffold. Cell accumulation is indicated with yellow arrow. B shows the abluminal view of the SIS scaffold containing crossed straight fibers.

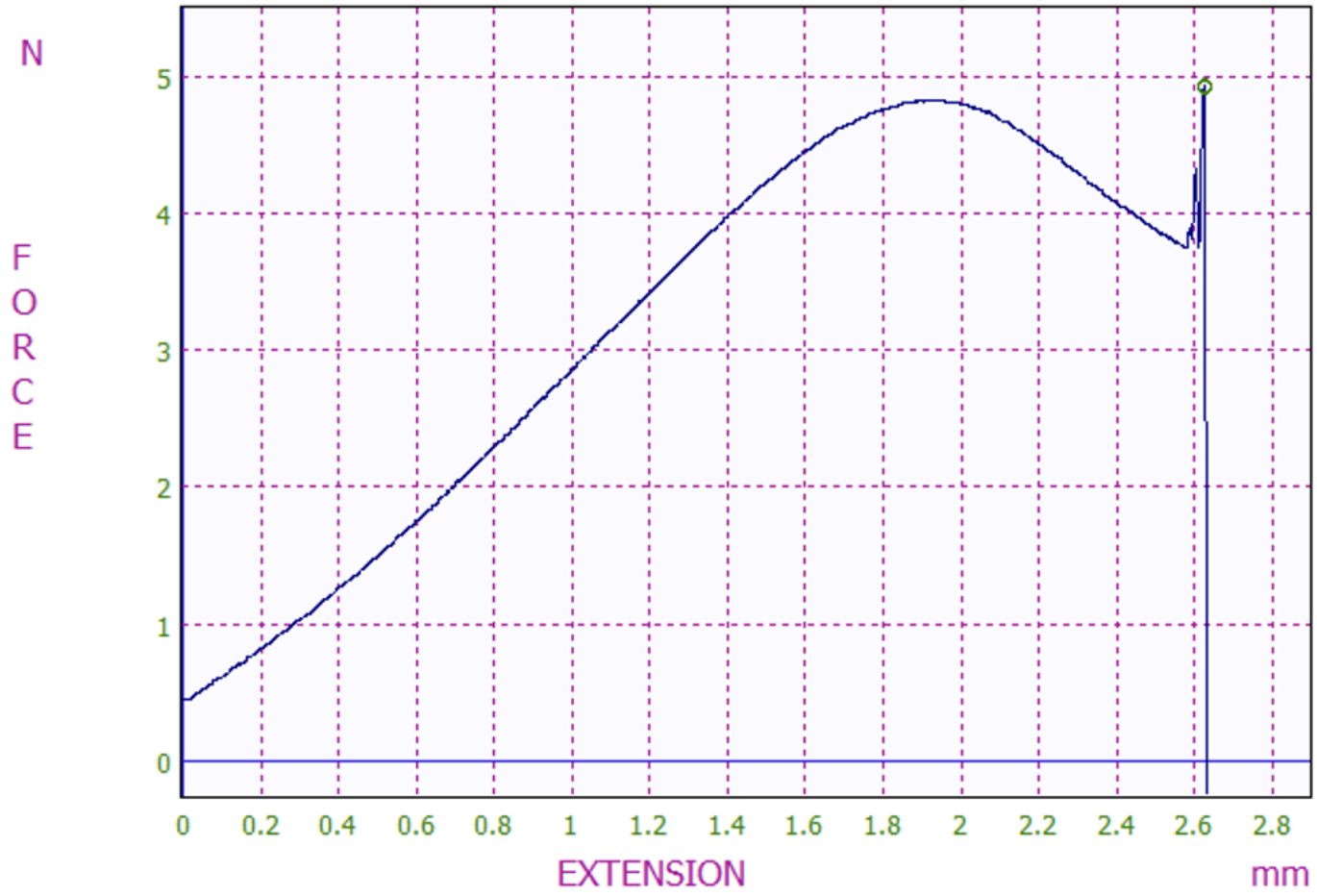
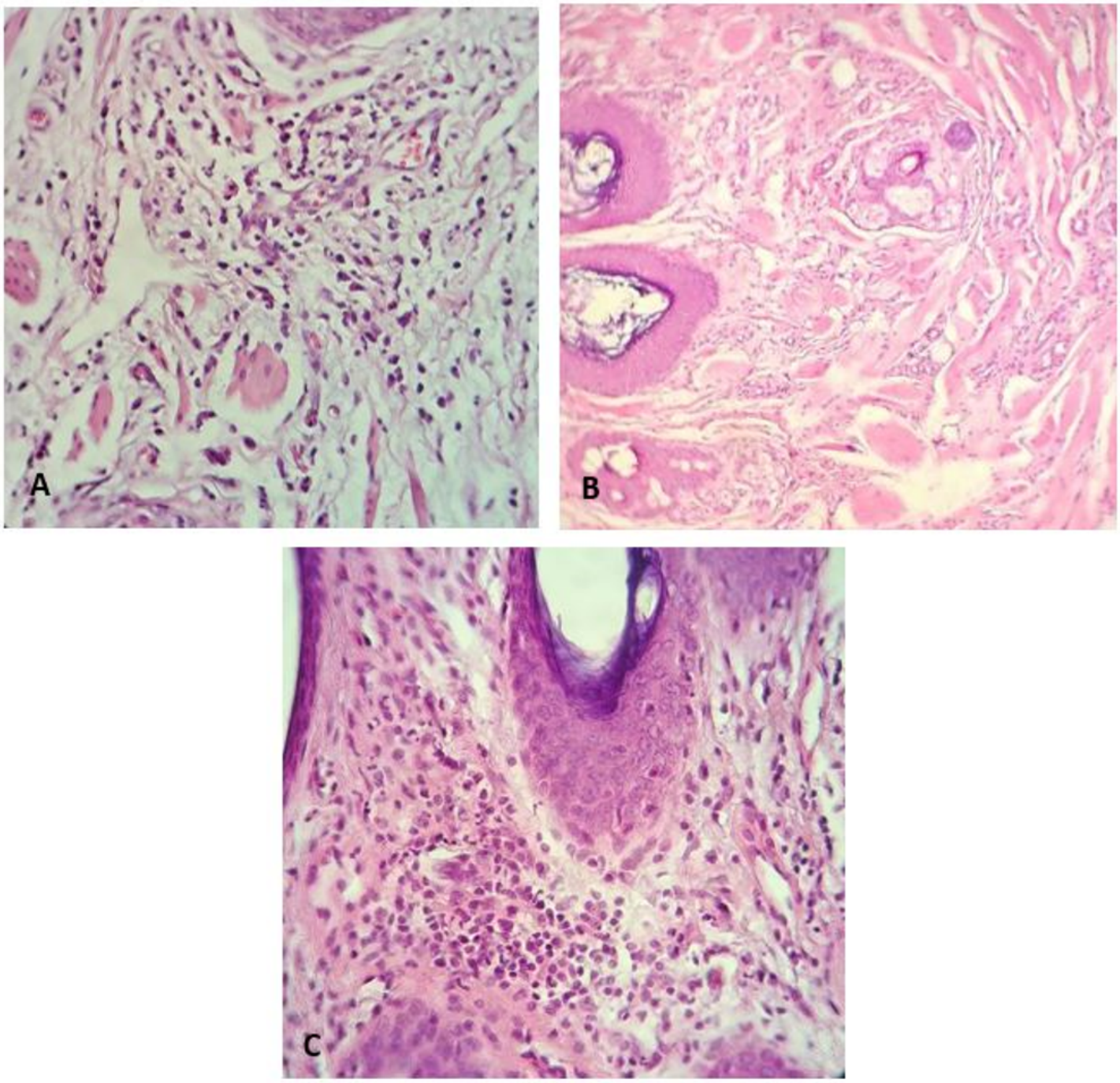



**Figure 4**

**load-elongation curve in an SIS sample**

Tensile strength in this sample peaked at 4.80 N and in 1.84 mm elongation.




## Figure 5

Histological analysis of inflammation in fistula

Moderate inflammation can be observed in SIS group (A), mild inflammation in SIS-PRF group (B) and severe inflammation in control group (C).