Journal Section: Reviews

Treatments for the Amelioration of Persistent Factors in Complex Anal Fistula

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Abstract

Anal fistulae are abnormal hollow connections between the wall of the anal canal and the perianal skin around the anus that have remained a burden on the medical sector for centuries. The complexity of this disease is attributed to a number of factors such as the degree of associated sphincter muscle, concomitant illnesses, existence of multiple fistulous tracts and the number of previous interventions. Persistence of a complex anal fistula can cause a decline in patient's physical quality of life as well as impact on the psychological status of patients who often suffer from anxiety and depression.

Surgical intervention remains the gold standard for treatment, however; the risk of incontinence and high recurrence potential has led to interest into developing alternative treatment approaches such as the use of biologics, bioactives and biomaterials. One potential reason for these varied outcomes could be the multifactorial interplay between genetic, immune-related, environmental, and microbial persistence factors on tissue regeneration. Recent observations have proposed that adverse inflammatory mediators may contribute more than microbial factors. Augmentation of conventional surgical procedures with novel advances such as stem cell therapies and biomaterial scaffolds are hoped to ameliorate persistent factors while facilitating a means to closing the fistula tract. The purpose of this review is to outline recent advances in biologics and combination therapies to treat persistent factors associated with complex anal fistula.

Introduction

Anal fistulae are prominent anal diseases that have remained a burden on the medical sector for centuries. An anal fistula is an abnormal hollow connection that protrudes from the wall of the anal canal to the perianal skin of the anus. Anal fistulae can manifest in various forms as shown in Fig 1. along the path of least resistance, some of which are deemed more complex than others.

This complexity can be attributed to a number of factors such as the degree of associated sphincter muscle, concomitant illnesses, existence of multiple fistulous tracts and the number of previous interventions.

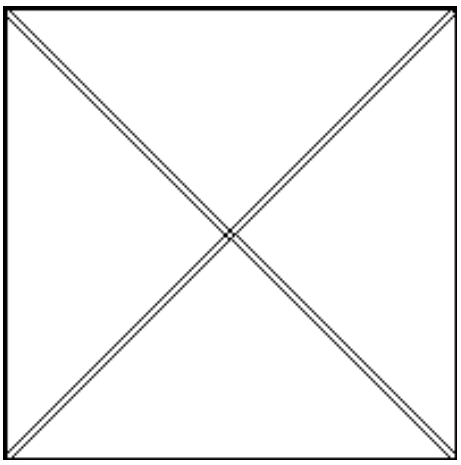


Figure 1. Anatomy and associated complexity classification of the anal fistulae

Regardless of whether these diseases are associated with a concomitant illness such as Crohn's disease or irritable bowel disease; Michelassi, et al (2000) attributed the decline in patient's quality of life to *"the associated pain, embarrassing discharge as well as psychological and sexual impairment"* (Michelassi et al., 2000). Patients with Crohn's disease are 25-40% likely to develop anal fistulae, the majority of which are complex (Castro-Poceiro et al., 2018). The manifestation of anal fistula has been reported across Europe with varying incidence rates of 10.4 per 100,000/year in Spain to 22.2 per 100,000/year in Italy. The prevalence of anal fistula is not known due to the 26-38% association with anal abscesses (Panés & Rimola, 2017; Sainio, 1984; Zanotti et al., 2007). Anal fistulae are noted by Lundqvist et al, (2016) as costly with regard to health care resources and sick leave. Costs can vary greatly depending on the degree and number of interventions required but it is not noted whether patients healed sufficiently.

With an estimated incidence rate of 13/100,000/year in Sweden; the financial burden to the medical sector would equate to 5 – 7 million euros (Lundqvist et al., 2016).

Fistulotomy is well cited in the literature as the ideal treatment for superficial anal fistula with success rates greater than 90% (Göttgens et al., 2015). Fistulotomy is not an ideal procedure for complex anal fistula as it may lead to cutting through the internal and external sphincter. Impairment in the functionality of the anal sphincters, known as incontinence, has led to the development of several sphincter-preserving operations in order to retain sphincter functionality while closing of the fistula tract (Dudukgian & Abcarian, 2011). The recurrence of anal abscesses and postoperative anal fistula formation rates between 30% - 50% even when microbial contents are sufficiently drained (Gokce et al., 2020). The complexity of anal fistula as described by Narang et al. (2017), dictates the potential intervention options available and has led to an increasing interest into the applicability of biologics, bioactives and biomaterials for treating complex anal fistulae (Narang et al., 2017).

Microbiota and Antibiotics

The microbiota of anorectal abscesses and fistula is similar in that they both consist of enterobacteria with *E.coli* accounting for a large portion of the microbial content (Eykyn & Grace, 1986; Ulug et al., 2010). Microbiota profiling of anal abscesses has been suggested by Dowling Enez & Izarra Henriquez, (2020) as a means of predicting the development of an anal fistula. The authors note that the isolation of anaerobic bacteria was observed in all samples, of which 80.9% manifested into an anal fistula within 3 weeks post-surgical drainage.

The dominant nature of *E.coli* observed in fistulized patients allowed the authors to infer it as a predictive factor of fistula development; most typically transsphincteric fistulae. Samples

presenting with polymicrobiota were observed to develop into complex extrasphincteric and suprasphinteric fistulae (Dowling Enez & Izarra Henriquez, 2020)

The existence of co-morbidities can increase the susceptibility to specific bacterial species. A retrospective study in Taiwan observed that *Klebsiella pneumoniae* was the most abundant microbe in patients with diabetes mellitus. In contrast, *E.coli* was the most abundant microbe isolated from patients without diabetes mellitus. (Liu et al., 2011). Recently, it has been observed by Jaiswal et al (2021) that there is significant (P = .054) abundance of enterobacteria capable of forming biofilms in persistent anal fistula. The authors note biofilm producing *E. coli* strains were statistically (P = 0.012) more predominant in anal fistulae persisting longer than 6 months (Jaiswal et al., 2021). Univariant and regression analysis in recent studies has shown that antibiotics are a protective factor against the manifestation of anal fistula (Brar et al., 2020; Ghahramani et al., 2017). A systematic review by Mocanu et al (2019) highlights the use of antibiotics as post-operative prophylactics to prevent the progression of an anal fistula. It was observed that postoperative antibiotic have a statistically significant preventative capacity of 36% against fistula development (P=0.03) (Mocanu et al., 2019). The role of peptidoglycan on the persistent inflammation of fistula tracts was investigated by van Onkelen et al (2013). The low concentration of bacteria observed in this study implied that bacterial infection does not contribute a major role in the persistent inflammation. In contrast, an immunostimulation effect was observed in response to peptidoglycan exposure. The secretion of interleukin-1β and other inflammatory mediators are highlighted as potential elements of the persistent inflammation (van Onkelen et al., 2013).

A subsequent study Tozer, et al. (2015) observed similar results in anal fistulising Crohn's disease showing that anal fistulae do not harbor an abundance of bacteria. However, evidence

was found of an effective host defense against bacterial subunits such as lipopolysaccharides. Embedding of these subunits in immunological cells may provide a mechanism for the adverse inflammation observed by van Onkelen et al. (2016). These findings suggest that cytokines such as interleukin 1β may contribute to the inflammatory process of complex anal fistula (Tozer et al., 2015; van Onkelen et al., 2016). Further explanations are required to understand the degree of significance microbial factors have on the persistence of anal fistulae (Sugrue et al., 2017).

Persistent Inflammation

Tissue regeneration and inflammation have been shown to act as a double-edge sword. Acute inflammation is required for tissue repair, whereas chronic inflammation can inhibit regeneration (Hasegawa et al., 2017). In terms of anal fistulae; the presence of granulation tissue has been observed as indicative of an inflammatory response which may be an attributing factor to the poor regeneration (Mitalas et al., 2012; van Koperen et al., 2010). Macrophages are vital components in the inflammatory response of tissue regeneration. These cells present pro-inflammatory and pro-healing phenotypes which can influence the progression of disease (Gaffney et al., 2017). To address the adverse inflammatory mediators observed in anal fistulae by Tozer et al (2015) and van Onkelen et al (2016), several interventions have been implemented.

Curcumin

The wound healing capacity of curcumin has been attributed to its anti-inflammatory and antimicrobial properties (Akbik et al., 2014). The application of curcumin for anal fistulae is scarce in western society but widely advocated for in societies that implement the Ksharasutra style of medicine (Mohite et al., 1997). A major set back for the use of curcumin for anal fistula is it's low bioavailability, which is typically mitigated by using higher non-toxic doses (Anand et

al., 2007; Kunnumakkara et al., 2019).. To overcome this issue, Xie et al (2018) integrated curcumin into a silk scaffold for anal fistulae. The steady drug release and cytocompatibility detailed are characteristics that show optimism for anal fistulae clinical trials (Xie et al., 2018). Another promising agent is a novel derivative of curcumin, Theracurmin®, has been observed to have a 27-fold increase absorption rate than natural curcumin. While investigating the effects of Theracurmin® on Crohn's associated anal lesions (fistulae and fissures), Sugimoto et al (2020) observed greater healing in treated patients at all time points. However, only the results at 8 weeks showed a significant ($p = 0.017$) improvement over the placebo group (Sugimoto et al., 2020). Further studies are required to observe the efficacy of curcumin on anal fistulae healing but initial results are promising.

Basic fibroblastic growth factor

Basic fibroblastic growth factor (bFGF) is a potent mitogen for cells in the formation of granulation tissue and tissue regeneration. Early studies have shown promise for bFGF as a treatment strategy for anal fistula but additional studies in adult populations are required (Ge & Guo, 2002; Kubota et al., 2010). The short shelf-life of bFGF may impair its ability to induce sufficient tissue regeneration while in-situ. To address this limitation, controlled release of bFGF has been shown to promote the proliferation of fibroblasts and provide sufficient regeneration. This delivery system has yet to be applied to anal fistula (Huang et al., 2016; Peng et al., 2020).

Mesenchymal stem cell

Injection of mesenchymal stem cells (MSC's) is a novel technique that has shown promise for treating perianal fistulizing Crohn's disease; especially patients who do not respond well to biologics such as infliximab, vedolizumab and Ustekinumab. The success of stem cells may be mediated by the anti-inflammatory and immune-regulatory properties more so than by their

ability to engraft and differentiate into the proximal tissue. The ability to produce and release mediators such as chemokines, complement factors, immunosuppressive molecules, growth factors, exosomes, and metabolites may contribute to the success of stem cell therapies (LeBlanc, 2008; Theodoropoulos et al., 2019). Biotechnological advance by TiGenix/Takeda have led to the development of Alofisel (darvadstrocel, CX601; TiGenix/Takeda); a suspension of allogeneic expanded adipose-derived stem cells. This product has recently been approved in the European Union for the treatment for anal diseases (Meng & Baumgart, 2020). While determining the efficacy and safety of Alofisel, Panés et al (2018) observed that 59.2% of patients showed the absence of draining fistulae at week 52. However, throughout the study period, 76.7% of patients in the Alofisel group and 72.5% patients in the control group experienced treatment-emergent adverse events (Panés et al., 2018).

More recently, Herreros et al (2019) assessed the potential of adipose-derived stem cells in patients that had previously undergone multiple surgical interventions. Crohn's and non-Crohn's associated anal fistulae showed healing of 55.5% and 50% respectively (Herreros et al., 2019). To address the moderate success rate of stem cell interventions alone, Dietz et al (2017) constructed an augmentation of autologous stem cells and a biomaterial plug. In this small cohort study of 12 patients, successful closure was observed in 83% of patients. (Dietz et al., 2017). A subsequent meta-analysis conducted by Cao el at (2021), observed higher healing rates and lower incidence of adverse events in patients given a stem cell therapy than those in the placebo group. Application of stem cell therapies in patients of fistulizing Crohn's disease is showing promise but remains in its infancy at present (Cao et al., 2021). The preparation and use of such stem cell therapies is noted by Dige et al. (2019) as time consuming and expensive which could hinder their use if success rate remain moderate (Dige et al., 2019).

Platelet rich plasma

The ability to be readily isolated and prepared by centrifugation, platelet rich plasma (PRP) has been shown as a low cost implement in healing complex anal fistulae. PRP consists of an array of bioactives, such as growth factors, peptides, and cytokines that stimulate tissue regeneration. Attractive features of PRP are its ability to enhance tissue regeneration as well as the lack of problems associated with immunogenicity (Samadi et al., 2019).

A multicenter study observed 51.66% complete recovery through primary augmentation of PRP with resecting and curettage. Fifteen patients with persistent fistulae were treated with a second dose of PRP and ten of these patients achieved full recovery. Accounting for both cohorts, a total of 68.33% of patients recovered (Pérez Lara et al., 2015). Similar results were observed by Moreno Serrano et al. (2016) in patients treated with platelet rich fibrin; a second generation PRP product that utilizes a fibrin scaffold to support the potent growth factors. The authors describe asymptomatic patients as a surgical success even when a persistent tract is observed by MRI. Among the 21 patients included in this study 61.9% were deemed a success while 31% experienced recurrence attributed to persistent factors (Moreno Serrano et al., 2016).

Pérez Lara and colleagues observed promising healing rates from an augmentation of PRP and the use of a novel curette technique. Curetting greater amounts of proximal fibrous tissue was hypothesized to improve the success outcome of PRP in anal fistulae. Healing rates ranging from 52.9% to 80.7% were observed, with the latter observed in the cohort that underwent both PRP and novel curettage procedure (Pérez Lara et al., 2019). When comparing the healing capacity of PRP and fibrin for anal fistulae, de la Portilla et al (2019) observed that PRP was as effective as fibrin glue. In contrast to previous literature, a successful healing was achieved in 48.4% and 41.7% of cohorts respectively (de la Portilla et al., 2019). A subsequent study investigating the

use of PRP in fistulising Crohn's disease reported a similar 40% complete healing rate after 48 weeks. The authors note that although the overall closure rate is lower than that of MSC therapies; PRP may facilitate greater accessibility and feasibility (de la Portilla et al., 2020).

Although results are promising, there remains many contributing factors to the efficacy of PRP. Sonker and Dubey (2015) note that each PRP preparation may differ with regard to platelet concentration, activation rates, and the profile of growth factors (Sonker & Dubey, 2015). Lei et al. (2019) attribute the lack of standardised methodologies for PRP preparation and utilization to the difficulty in assessing efficacy between cohorts (Lei et al., 2019).

Bioresorbable polymers

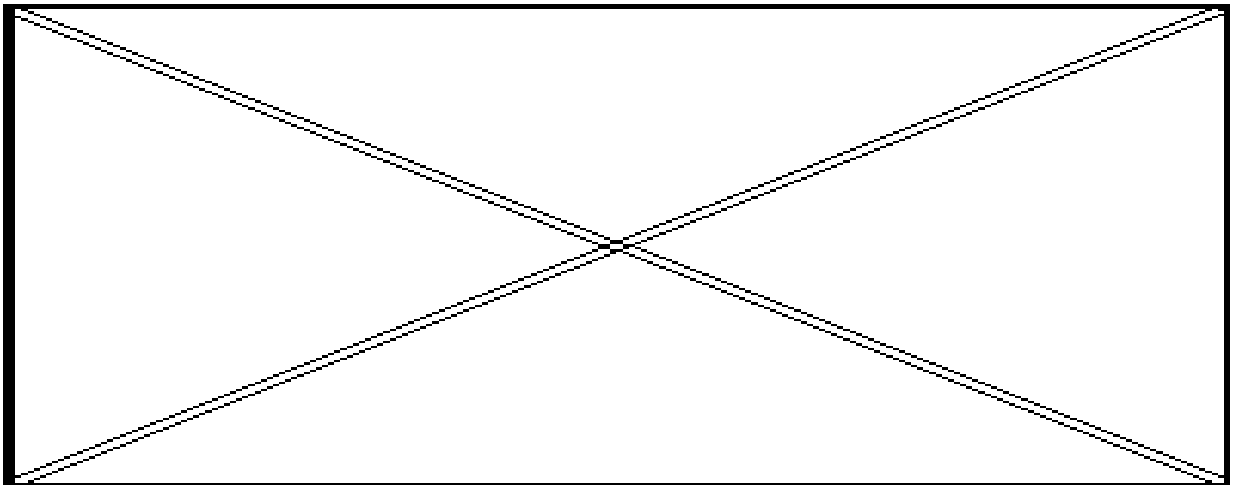
Tissue engineering relies on scaffolds that mimic the injured/damaged area that needs to be repaired or even replaced (Langer & Vacanti, 2016). Advances in collagen processing in the early 2000's that retain the composition of the extracellular matrix facilitated the development of the first anal fistula plug (Hiles et al., 2009). Collagen remains promising for anal fistulae as it complies with the Vroman effect while acting as a bioresorbable scaffold.

With over a decade in clinical practice, success rates with crosslinked Permacol™ held promise initially but subsequent studies shown varied outcome respective of follow-up time (Bayrak & Altıntas, 2018; Giordano et al., 2016). Recently, the development of a non-crosslinked collagen matrix by Maternini et al. (2020) observed similar results to those of early applications of Permacol™ (Hammond et al., 2011). The non-crosslinked matrix facilitated 78.5% (n=70, 55/70) of patients achieving clinically healed fistulae (Maternini et al., 2020).

The application of bioresorbable polymers has become an area of interest for clinicians as a scaffold material but also a means of overcoming the limitations of biologics.

(Aho Fält et al., 2020; McGee & Champagne, 2009; Ratto et al., 2012). Interestingly, Blaker et al (2008) developed microspheres with an integrated antimicrobial delivery system as a means of guided tissue regeneration while providing localized delivery. Inhibition of microbial growth and infiltration of fibrovascular tissue shows promise for this drug delivery scaffold (Blaker et al., 2008; Keshaw et al., 2010). The first-in-human feasibility study of these TIPS microspheres is currently underway with 2022 set as the completion date (University College, London, 2020)

Table 1. Summary of novel advances for the treatment of complex anal fistulae (CAF)



Conclusion

Biologic interventions have advanced to address the multifactorial interplay of anal fistula persistence and its tissue regeneration. Low populations of biofilm producing bacteria suggest disease persistence may be primarily impacted by the presence of adverse inflammatory cytokines such as IL-1β. In order to ensure proper closure of the fistula tract, must we disassociate between factors attributed to the manifestation and persistence of the disease?

Stem cells and platelet rich plasma show promise as sources for adapting the adverse environment of anal fistulae but results remain in their infancy. Application of bioresorbable

polymers has been increasing due to the issues with current biostable implants, conducting revision surgeries and biologic durability.

Amelioration of persistent factors may facilitate a means of stabilizing the proximal tissue, thus allowing sufficient tissue regeneration. Going forward, augmentation therapies consisting of minimally invasive surgical procedures, biologics and biomaterials may become the next generation in the treatment of complex anal fistulae.

Conflict of Interest / Competing interests

The authors disclose that there are no conflicts of interest

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Authors' contributions

Conceptualization, D.F.; methodology, D.F.; data, D.F.; writing—original draft preparation, D.F; writing—review and editing, D.F., C.K, D.B, M.G and N.G.; supervision, C.K, D.B, M.G, N.G.

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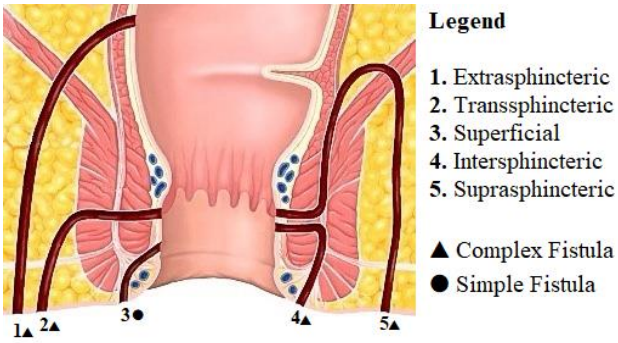

Figure 1 Anatomy and associated

complexity classification of the anal fistulae

Table 1. Summary of novel advances for the treatment of complex anal fistulae (CAF)

Intervention	Success Rate	Advantages	Limitations	Reference
Antibiotics	36% – 50%*	Prophylactic Cost benefit Favorable safety profile	Ineffective as sole treatment for CAF*	(Mocanu et al., 2019) (Dejaco et al., 2003)
Curcumin	35% – 40%	Favorable safety profile Localized drug delivery capability	Innate bioavailability Insufficient published literature for CAF Not adopted into western practices	(Sugimoto et al., 2020) (Xie et al., 2018)
Stem Cells	50% - 83%	Addresses multifactorial	Expensive Time consuming	(Dietz et al., 2017; Dige et al., 2019;

		interplay of CAF		Herreros et al., 2019)
Basic fibroblastic growth factor	N/A**	Potential stand-alone product	Short shelf life Insufficient published literature in adult populations	(Ge & Guo, 2002; Kubota et al., 2010)
Platelet Rich Plasma	40% - 61.9%	Ease of preparation	Costly processing systems	(Moreno Serrano et al., 2016)
		Minimally invasive Addresses multi-factorial interplay of CAF	Costly if repeated interventions required	
Fibrin Glue	23% - 80%	Simple procedure Minimally invasive	Poor long-term healing Contraction within	(Almeida et al., 2018; Cirocchi et al., 2009;

			FT Low reproducibility in patient derived fibrin glue	Loungnarath et al., 2004)
Permacol™	47.6% - 57%	Simple procedure Minimally invasive Biocompatible	Low elasticity May require corrective surgery	(Brunner et al., 2019; Fabiani et al., 2017; Giordano et al., 2018; Schiano di Visconte et al., 2019)
Salvecoll-E-Gel	78.5%	N/A**	N/A**	(Maternini et al., 2020)
TIPS Microspheres	N/A**	Localized drug delivery Sustained release	Currently in clinical trial**	(Blaker et al., 2008)

CAF = Complex Anal Fistula | FT = Fistula Tract

*Percent probability of not developing an anal fistula after treating anal abscess

** Device is currently in clinical trial or not enough published literature