Am J Otolaryngol 40 (2019) 101–105

Contents lists available at ScienceDirect

Am J Otolaryngol

journal homepage: www.elsevier.com/locate/amjoto

In vitro analysis of growth patterns of invasive fungal species on commonly used endonasal hemostatic agents☆

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ARTICLE INFO

Keywords:
Invasive fungal sinusitis
Nasal packing
Hemostasis

ABSTRACT

Objective: Previous studies have not examined the potential role of endonasal hemostatic agents in facilitating growth of fungal species. We aim to determine the possibility of these to serve as a nutrient source for fungal growth.

Methods: Cultures of *Aspergillus*, *Fusarium*, and *Mucor* were harvested and placed in solution in sterile saline at standardized high and low concentrations. Thrombin gelatin matrix, carboxyl methylcelluose, and potato starch derivative agents were prepared following manufacturer instructions and applied to two separate Petri dishes per agent. Each substrate was then inoculated with either high or low concentrations of fungal species. Negative and positive control plates with each organism were included. Dishes were sealed, incubated, and examined daily for fourteen days for microscopic and macroscopic growth.

Results: Thrombin gelatin matrix was relatively resilient to growth, although *Fusarium* growth was noted on all packing material by day three. Carboxyl methylcellulose also supported growth of high-concentration *Mucor* appreciated on day five. The potato starch derivative supported fulminant growth of all fungal species.

Conclusions: Endonasal hemostatic agents may be nutrient sources that facilitate growth of fungal species. This may be a consideration in a surgeon's decision to use a hemostatic agent. Prompt initial post-operative debridement may be warranted in select patients. Our findings serve as a model for further testing of fungal growth on other hemostatic materials. Future studies are needed to confirm the clinical significance of these findings *in vivo*.

1. Introduction

Invasive fungal rhinosinusitis (IFRS) is a life-threatening infection of the nose and paranasal sinuses. These infections can be subdivided into acute and chronic forms, both with differing patient demographics and prognoses. However, the mainstay of treatment for any form of invasive fungal sinusitis is similar and consists of surgical debridement of necrotic tissue alongside long-term antifungal therapy [11,12]. When performed, debridement should proceed until normal bleeding tissue is encountered [1]. Acute IFRS (AIFRS) typically affects immunocompromised patients and may be rapidly fatal if not diagnosed early and aggressively debrided. The most common fungal species identified in acutely invasive infections are *Aspergillus* and *Mucor* [2,3]. Dematiaceous fungi have been implicated in some cases of AIFRS as well [4]. Many patients that develop AIFRS have an underlying comorbid acute hematologic malignancy and have been treated with chemotherapy and/or may have undergone bone marrow transplantation [5]. All of these factors predispose these patients to pancytopenia, blood dyscrasias, and significant bleeding diatheses. Combining this predisposition with the need for aggressive debridement places this particular subset of patients at substantially increased risk of postoperative hemorrhage. Dissolvable endonasal hemostatic packing material may, therefore, be beneficial and reasonable to use in these patients. However, many of these products have organic derivatives, some of which are comprised partly of potato starch, a routine component of laboratory fungal growth media. With this in mind, we hypothesized

☆ Meeting Information: Oral Presentation on January 19, 2018 at the 2018 Triological Society Combined Sections Meeting in Scottsdale, AZ, United States.
Conflicts of Interest: The senior author, S.E.K., serves on the scientific advisory board of Optinose and on the surgeon advisory board of Hemostasis, L.L.C. The authors have no other disclosures.

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https://doi.org/10.1016/j.amjoto.2018.09.016
Received 11 September 2018

that these agents may actually serve as a growth medium for invasive fungal species. The goal of this study is to evaluate the *in vitro* fungal growth potential of 3 different fungal species on 3 commonly employed endonasal hemostatic dressings.

2. Methods

This study was exempt from Institutional Review Board approval. Endonasal hemostatic packing material samples were donated by third party vendors. The three hemostatic materials used were Nexstat (Hemostasis, LLC, St. Paul, MN, USA), Sinu-foam (Smith & Nephew, Heslington, York, UK), and Floseal (Baxter Healthcare Corporation, Hayward, CA, USA). Nexstat is a topical hemostatic powder comprised partially of potato starch. It is applied through a flexible applicator to the sites of interest. It has sister products, such as Nexfoam, that are more cohesive but similar in composition. Sinu-foam is a carboxyl methylcellulose ribbon that forms a viscous gel when mixed with water. Floseal (thrombin gelatin matrix) has a bovine-derived gelatin component that is mixed with a human-derived thrombin component and applied to the site of bleeding.

Aspergillus, *Fusarium*, and *Mucor* cultures were grown for 2–7 days in potato dextrose agar at 35 °C until sufficient sporulation was obtained. 1 mL of sterile 0.85% saline was placed onto the fungal colony and gently rubbed to loosen fungal conidia. The resulting solution was removed and transferred to a sterile tube. The solution was allowed to settle for 3–5 min and the upper homogenous mixture was transferred to a new sterile tube and vortexed for 15 s. The densities of the suspensions were measured using a spectrophotomer at 530-nm wavelength and adjusted to an optical density of 0.09–0.13 for *Aspergillus* spp. and 0.15–0.17 for *Mucor* spp. and *Fusarium* spp. To verify inoculum concentration, 0.01 mL of a 1:10 dilution was plated on Sabouraud dextrose agar and incubated at 30 °C. Colonies were counted as soon as growth was visible.

Conidial suspensions of an approximate concentration of 0.4×10^6 to 5×10^6 CFU/mL (high concentration) were made in sterile saline. This suspension was diluted 10^{-4} for the low concentration. Hemostatic agents were packaged in single units intended for one-time use. One unit of each hemostatic agent was prepared following manufacturer instructions and applied to two separate empty Petri dishes per agent. Each substrate was then inoculated with either high or low concentrations of fungal species. A negative control potato dextrose agar plate without inoculum and positive control potato starch agar plates inoculated with each organism were included (Figs. 1–3). Dishes were sealed, incubated, and examined daily for fourteen days using a microscope to assess for growth. The potato starch derivative test plates were white and opaque in color, making growth assessment difficult; therefore, tape preparations with lactophenol aniline blue stain were utilized in this group regularly to inspect for growth. Dishes remained sealed during inspection to maintain sterility unless a sample from the dish was required for staining to confirm growth. Seals were only broken to obtain tape preparations for lactophenol aniline blue dye staining once gross growth was noted in order to avoid premature breaches in sterility. Samples were obtained using sterile procedure in a biosafety cabinet.

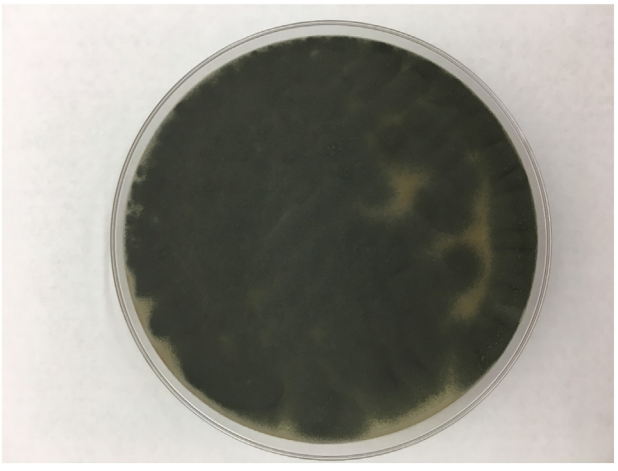

Fig. 1. Aspergillus control plate.

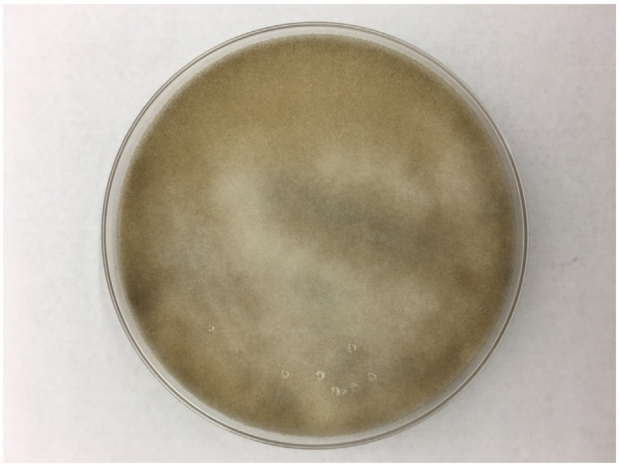

Fig. 2. Fusarium control plate.

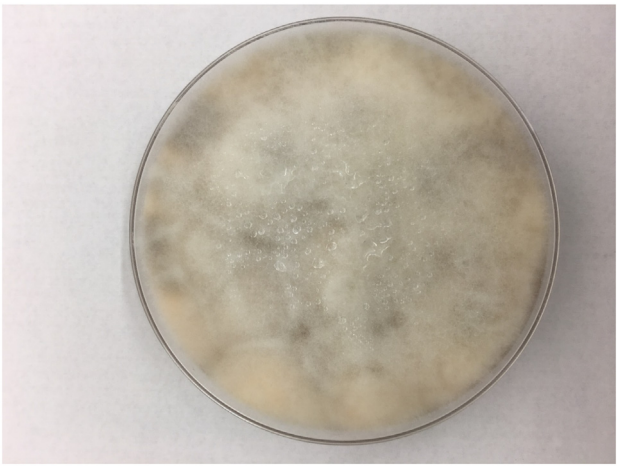

Fig. 3. Mucor control plate.

The scope of this study was to test the capability of the dissolvable packing materials used to support fungal growth. A dichotomous qualitative system was employed, noting whether growth was, or was not, supported. It was determined that a quantitative measure, such as measurement of colony-forming units, was not necessary given its difficulty and the lack of necessity to this study given our stated goals.

3. Results

All materials with organic derivatives sustained growth of fungal species. *Fusarium* had the most prominent growth of the species tested. The potato starch derivative allowed significant growth of all 3 fungal species tested, while the thrombin gelatin matrix was relatively resilient, with the exception of *Fusarium* growth using the high-concentration inoculum.

The potato starch derivative supported high-concentration inoculum *Aspergillus* (AH) growth appreciated grossly on day 3 (Fig. 4) and low-concentration inoculum *Aspergillus* (AL) on day 5. *Aspergillus* growth was seen as a dark colony in the media and was confirmed using

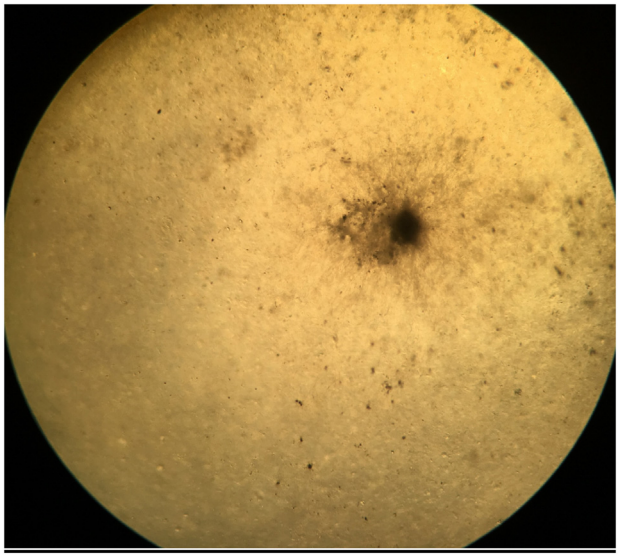

Fig. 4. Microscopic view of high-concentration *Aspergillus* inoculum on potato starch derivative Day 3.

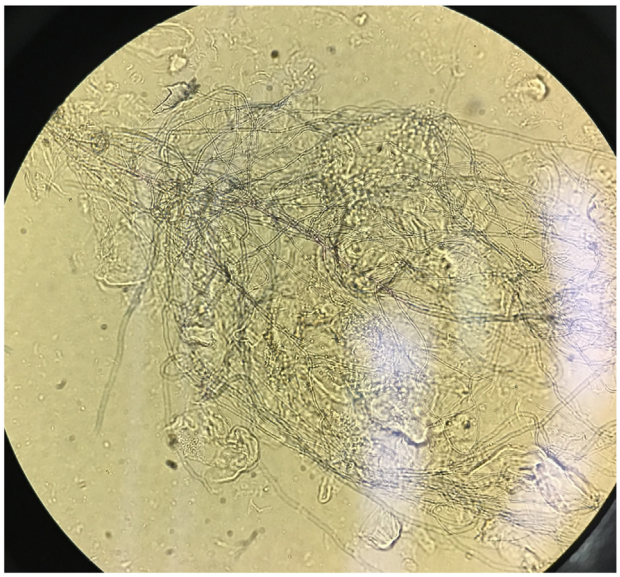

Fig. 5. Aniline blue dye confirmation of growth of low-concentration *Aspergillus* inoculum on potato starch derivative on Day 5.

the lactophenol aniline blue stain (Fig. 5). On day 5, low-concentration *Fusarium* (FL) and high-concentration *Fusarium* (FH) growth were appreciated. In both samples, growth was subtle and was evidenced by increased viscosity of the liquid media and matrix formation (Fig. 6). These findings prompted use of the lactophenol aniline blue stain (Fig. 7) for confirmation, and they suggest that earlier growth may have occurred undetected. Growth of high-concentration *Mucor* (MH) was also found microscopically on day 5, while low-concentration *Mucor* (ML) was appreciated on day 7 (Fig. 8).

Carboxyl methylcellulose supported growth of FH on day 3 (Fig. 9) and FL seen microscopically on day 5. *Fusarium* grew on the surface of carboxyl methylcellulose in a lattice pattern (Fig. 10). There was questionable microscopic growth of MH on day 5. This was seen under low power magnification on the fringe of the media and a *Mucor* filament with early budding was seen under high power magnification after aniline blue dye staining (Fig. 11). However, there was no further macro- or microscopic growth on this media through to the study end date at 14 days post inoculum. This may have represented organisms

Fig. 6. Matrix formation of high-concentration *Fusarium* inoculum on potato starch derivative on Day 5.

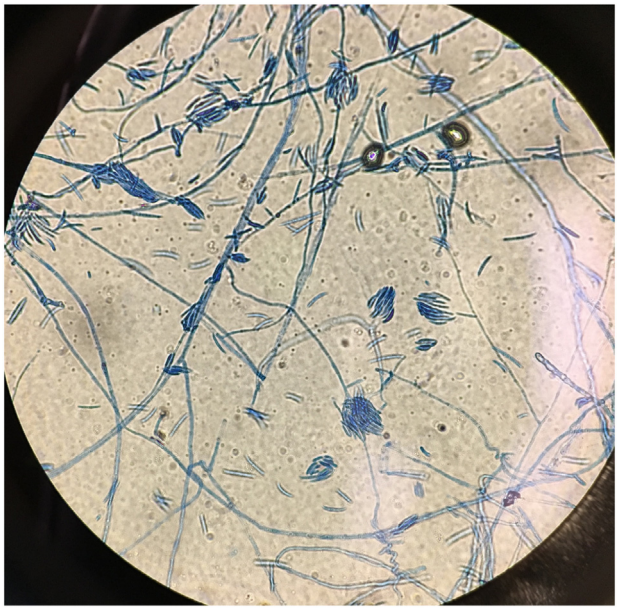

Fig. 7. Lactophenol analine blue dye sampling of control *Fusarium* colony.

from the inoculum itself or very mild, and likely clinically insignificant, growth.

Thrombin gelatin matrix exhibited growth of FH on day 3 (Fig. 12). No other organism at any concentration appeared to have growth on this substance; however, the granular surface of the material made it difficult to evaluate microscopically under high-power magnification and with the aniline blue fungal stain.

4. Discussion

This study demonstrates that starch-based endonasal hemostatic materials facilitate growth of invasive fungal species. Given the relative

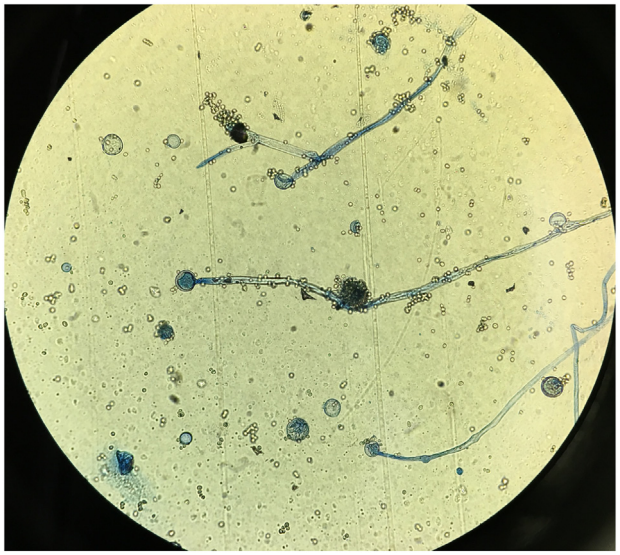

Fig. 8. Aniline blue dye confirmation of growth of low-concentration *Mucor* inoculum on potato starch derivative on Day 7.

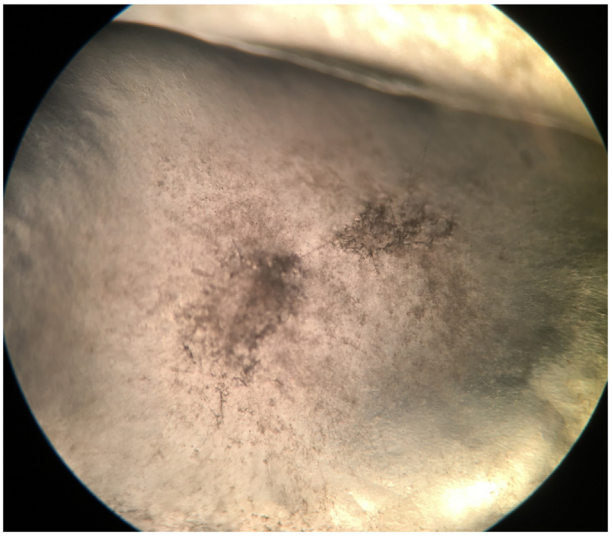

Fig. 9. Macroscopic growth of high-concentration *Fusarium* inoculum on carboxyl methylcellulose on Day 3.

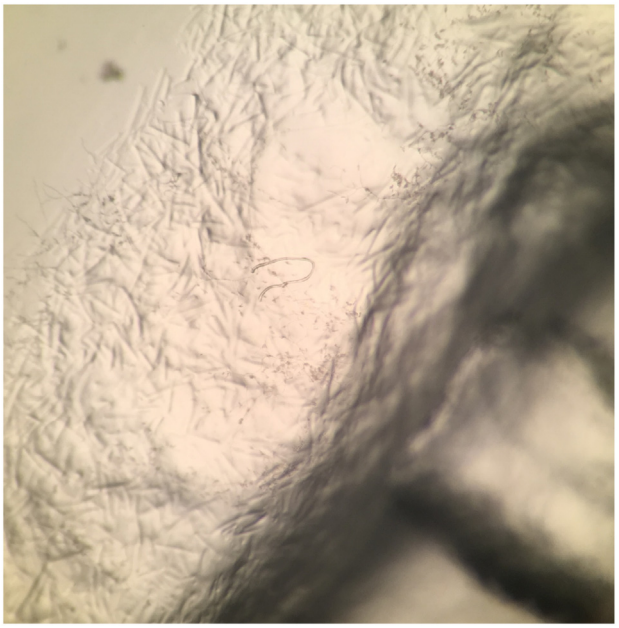

Fig. 10. Microscopic growth of low-concentration *Fusarium* inoculum on carboxyl methylcellulose on Day 5.

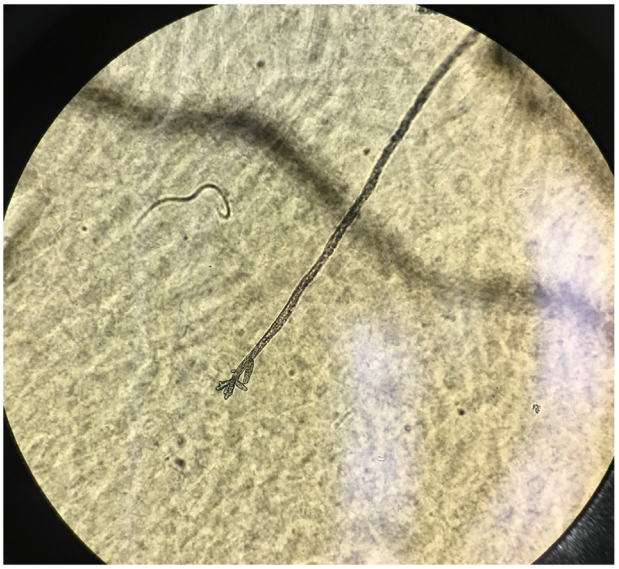

Fig. 11. Aniline blue dye confirmation of microscopic growth of *Mucor* inoculum on carboxyl methylcellulose on Day 5.

lack of growth on the carboxyl methylcellulose and thrombin matrix, these agents appear favorable for use in cases of invasive fungal disease where topical hemostatic agents may be employed. A caveat is that *Fusarium* appeared to have early growth on all 3 materials.

In the past, non-dissolvable nasal packing was used often for achieving hemostasis following sinus surgery, but this method has fallen out of favor due to patient discomfort and associated complications, such as toxic shock syndrome, dislodgement and aspiration, and nasal septal perforation [6,7]. As dissolvable hemostatic materials have entered the market, they have gained preference and offer patients improved quality of life in the early post-operative period compared to non-dissolvable packing [13]. However, the benefit of dissolvable biomaterials in preventing postoperative hemorrhage and scar formation remains controversial, and no single material has been proven to be superior [6,13–15].

Further studies have demonstrated a paucity of benefit in preventing post-operative hemorrhage when comparing endonasal packing groups to non-packed cohorts [9,10,13,17]. In fact, studies have shown increased scar formation when certain dissolvable hemostatic agents were used. One study evaluating Floseal showed it to be effective at hemostasis but found an increased incidence of scar formation with material incorporated into scar tissue during histopathologic examination [8]. Conversely, other recent studies have found fibrin glue and chitosan gel to be effective hemostatic agents, with chitosan gel also preventing post-operative scar formation [15,16].

It should be noted that each of these aforementioned studies mainly included patients without significant comorbidities or deficiencies that predispose them to a bleeding diathesis. Most patients susceptible to invasive fungal sinusitis are also at significantly increased risk of hemorrhage despite meticulous surgical technique due to their underlying co-morbidities. Patients with hematologic malignancies and bone marrow transplantation represent high-risk cohorts for acute invasive fungal sinusitis due to pancytopenia. These patients are thus faced with a predicament between requiring life-saving aggressive surgical

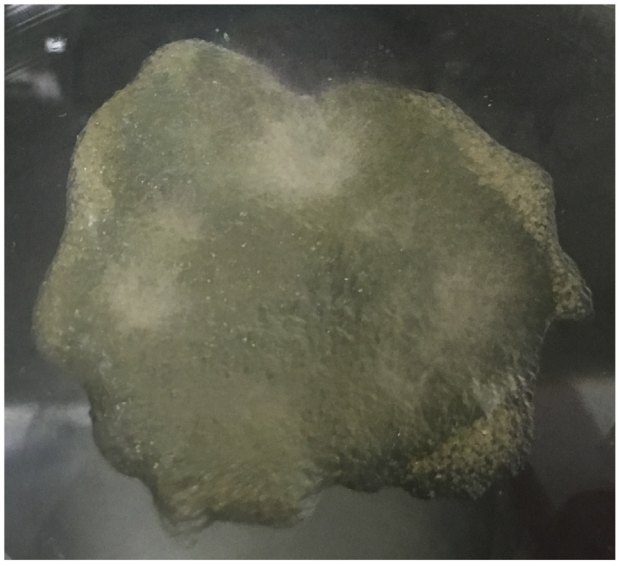

Fig. 12. Macroscopic growth of high-concentration *Fusarium* inoculum on thrombin gelatin matrix on Day 3.

debridement and a significantly increased risk of postoperative bleeding. At the time of this writing, there is no known study evaluating the need for hemostatic agents or packing in patients with thrombocytopenia or bleeding dyscrasias following endoscopic sinus surgery. Given the paucity of data on this topic, it is reasonable to employ a hemostatic agent following endoscopic sinus surgery for these patients.

While the *in vitro* nature of this study restricts its clinical significance, our findings do suggest that starch-based hemostatic materials may facilitate growth of fungal species. This potential is likely insignificant in the immunocompetent patient. However, use of starch-based packing agents in an immunocompromised patient in the setting of IFRS, may warrant early debridement by the sinus surgeon to prevent fungal growth. Future studies are needed to corroborate these findings.

5. Conclusion

Use of starch-based hemostatic agents following sinus surgery should be carefully considered in immunocompromised patients at risk for developing invasive fungal sinusitis and those undergoing debridement for invasive fungal sinusitis. If the operative surgeon deems use of a hemostatic agent to be necessary in these patients, non-starch materials are reasonable options. Our findings serve as a benchmark for further *in vitro* testing of fungal growth on other available topical hemostatic agents.

Acknowledgements

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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