Annals of Medical Research
DOI: 10.5455/annalsmedres.2018.09.189 2019;26(1):47-50

Original Article

Interaction between pancreatic fluid and three different hemostatic agents: An in-vitro study

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Abstract

Aim: Although recent developments in surgery led to mortality reduction under 2%, postoperative pancreatic fistula (POPF) remains high reported from 20% up to 40%. Primary aim of the present in-vitro study was to determine the interaction between pancreatic fluid and three different hemostatic agents.

Material and methods: Three different hemostatic agent; fibrin sealant Tisseel, Floseal and Ankaferd Blood Stopper (ABS) were mixed in tubes with pancreatic fluid in equal proportions. The length of the gel aggregate of each sample which covers the pancreatic fluid in the tube was measured as mm and thereafter statistically compared.

Results: Tisseel significantly formed an intensely thicker gel than Floseal and ABS (Tisseel vs FloSeal; P<0.0001, Tisseel vs ABS; P<0.0001). Further, the thickness of the gel formation was significantly higher in FloSeal-pancreatic fluid mixture than the ABS-pancreatic fluid mixture (P<0.0001). Under light microscope, Tisseel formed a much more homogenous and dens mixture than Floseal and ABS.

Conclusion: Tisseel fibrin sealant has beneath its hemostatic properties also the potential of preventing pancreatic fistula development. Further in-vitro and in-vivo studies are needed to reach a definitive conclusion.

Keywords: Hemostasis; Pancreatic Fistula; Hemostatic Agents; Interaction.

INTRODUCTION

Distal pancreatectomy is the treatment of choice for lesions localized to the body and tail of pancreas. Although recent developments in surgery led to mortality reduction under 2%, postoperative pancreatic fistula (POPF) remains high reported from 20% up to 40% (1-3). All effort and search are focused on prevention of the fistula development which in turn prolongs hospitalization and increases cost.

Various techniques and methods for the pancreatic stump closure have been described to solve this problem (4-10). Beneath the surgical technique and skill, presence of infection, and a soft tissue pancreas are the other risk factors that may have high probability on development of fistula formation (11-14).

Application of fibrin glues on pancreatic stump after the stump closure is a well-known method for preventing of fistula formation with controversial results (15-18). Further, either autologous patches or synthetic meshes such as polyglicolic acid are tried with the same purpose in a variety of studies (16,19). However, data lacks for a definitive conclusion regarding their efficacy.

The primary aim of the present in-vitro study was to determine the interaction between pancreatic fluid and three different hemostatic agents. Secondly, to realize which of these hemostatic agents may be applied on pancreatic remnant stump with the aim of preventing postoperative pancreatic fistula development (POPF).

MATERIAL and METHODS

The study was conducted in Sakarya University Training and Research Hospital. Three different hemostatic agent; fibrin sealant Tisseel® (Baxter, Deerfield, Illinois, USA), matrix hemostatic agent Floseal (Baxter HealthCare, Deerfield, Illinois, USA) and Ankaferd Blood Stopper (ABS, Ankaferd Health Products Ltd, Turkey) were mixed in tubes with pancreatic fluid in equal proportions.

Experimental Design

Twenty samples of each hemostatic agent were used

Received: 16.09.2018 **Accepted:** 12.10.2018 **Available online:** 17.10.2018
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for this experiment. Pure pancreatic fluid was collected from a patient who underwent pancreaticoduodenectomy for pancreatic head adenocarcinoma. The patient had developed grade C pancreatic fistula during his postoperative course. A second laparotomy was required for controlling sepsis in which the jejunal limb of the PJ anastomosis was closed. The pancreatic fluid was drained by an external drainage catheter inserted to the main pancreatic duct. The hemostatic agents were mixed in tubes with daily obtained pancreatic juice. One ml of pancreatic fluid was mixed with one ml of each sample of the hemostatic agents were in room temperature (24°C). The length of the gel aggregate of each sample which covers the pancreatic fluid in the tube was measured as mm and thereafter statistically compared (Figure 1). The mixtures were analyzed under light microscope with x10 and x40 magnifications.

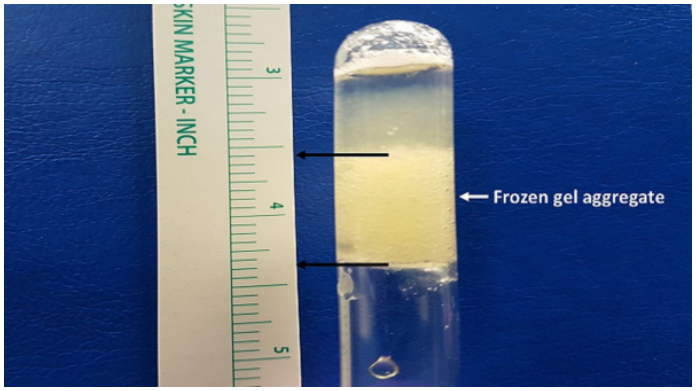

Figure 1. Measurement of the frozen gel aggregate as mm in inverted tube

Statistical Analysis

Data analysis was performed by using SPSS for Windows, (version 18, Chicago, IL, USA). One sample test and Kolmogorov Smirnov test was used to determine whether the distribution of continuous variables was normal or not. Continuous variables were shown as mean ± SD. To show the relationship between dependent groups binary, Paired sample t-test was used. A P value <0.05 was accepted as significant.

RESULTS

Twenty samples of each hemostatic agent were used for this experimental study. The mean thickness of the gel formation was 17.15±1.31 mm in Tisseel-pancreatic fluid mixture; 12.95±2.11 mm in Floseal-pancreatic fluid mixture, and 4.70±1.17 mm in ABS-pancreatic fluid mixture, respectively. When compared the thickness of gel formation, Tisseel significantly formed an intensely thicker gel than Floseal and ABS (Tisseel vs FloSeal; P<0.0001, Tisseel vs ABS; P<0.0001) (Figure 2). Further, the thickness of the gel formation was significantly higher in FloSeal-pancreatic fluid mixture than the ABS-pancreatic fluid mixture (P<0.0001), (Table 1).

The density of gel formation was evaluated under light microscope in x10 and x40 magnifications. When compared the mixtures, Tisseel formed a much more homogenous mixture followed by Floseal and ABS, respectively (Figure 3).

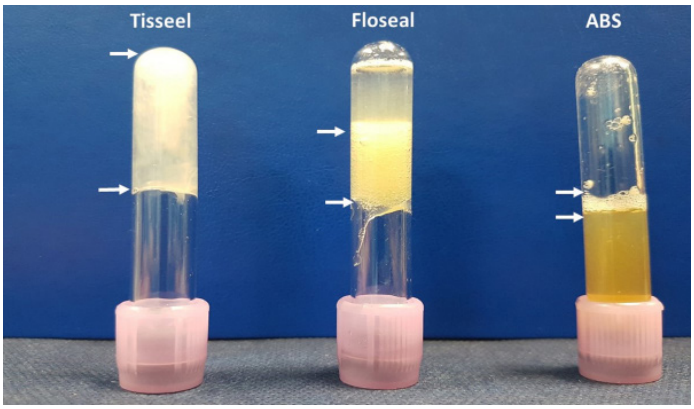

Figure 2. Comparison of the mixtures according to the gel formation in inverted tubes

Table 1. Comparison of the mixtures of hemostatic agents with pancreatic fluid according to gel formation

Mixture	The mean size of the gel formation (mm)	P Value
Tisseel (n=20)	17.15±1.31	<0.0001
Floseal (n=20)	12.95±2.11	<0.0001
ABS (n=20)	4.70±1.17	<0.0001

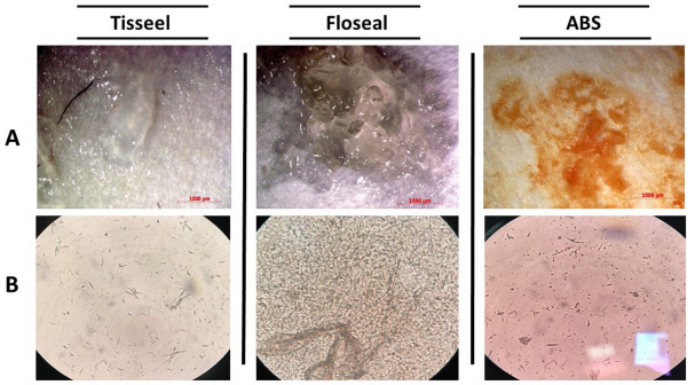

Figure 3. Light microscopy images of the mixtures. Comparison of the density of mixtures in A) x10 and B) x 40 magnifications

DISCUSSION

Daily, about 2.5 liters of clear, colorless, bicarbonate-rich pancreatic fluid containing 6 to 20 gr of protein is secreted from the human pancreas. With the exception of the lactating mammary gland, the exocrine pancreas synthesizes protein at a greater rate, per gram of tissue, than any other tissue. More than 90% of pancreatic proteins consist of secretary digestive enzymes (20), which have the greatest obstacle in tissue healing at the pancreatic stump cut surface or the pancreaticojejunostomy anastomosis due to their digestive properties with the high possibility of fistula development.

The main interaction principle of the hemostatic agents works whether by the coagulation cascade or by aggregation of the protein network. Tisseel fibrin sealant consist of human fibrinogen and human thrombin that mimics the final stages of the body's natural clotting

cascade but forms a cloth formation independent of the body's own coagulation cascade. One advantage of Tisseel is its applicability on heparinized patients with equal effectiveness. Application of Tisseel on gastric remnant surface during laparoscopic sleeve gastrectomy is a well-known purpose for achieving blood hemostasis and preventing leakage (21,22). Further, reproducible sealing effects of fibrin glue on the healing of gastrojejunal anastomoses has been previously demonstrated (23,24).

Floseal, a human gelatin-thrombin matrix sealant, provides a proprietary combination of two independent hemostatic agents. First, the gelatin granules swell to produce a tamponade effect, and secondly, high concentrations of human thrombin convert fibrinogen into fibrin monomers which accelerates cloth formation. A review which analyzed 27 reported studies, confirmed that Floseal® showed improvements over other hemostatic agents in achieving hemostasis and reducing blood loss (25).

ABS is a standardized herbal extract obtained from five different plants Thymus vulgaris, Glycyrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica [26]. In vitro and in vivo studies have shown that ABS promote the formation of an encapsulated protein mesh which acts as an anchor for erythrocyte aggregation out of the coagulation cascade (27,28). It has been previously showed that application of ABS on pancreatic fluid forms aggregates of protein network as the same way as in blood homeostasis (29,30).

This is the first in-vitro experimental study which compares the interaction between pancreatic fluid and three different hemostatic agents. Results of the present study demonstrated that Tisseel is significantly more effective than the other two hemostatic agents in creating of a frozen gel matrix and has the potential of preventing of fistula formation by application on the pancreaticojejunostomy anastomosis or the remnant pancreatic stump.

The nutritional status of the patient may affect the formation of the gel matrix in relation with the amount of pancreatic protein. In malnutrition states with low albumin levels, the efficiency of the three hemostatic agents may decrease.

The present study has some limitations. First it's an in-vitro study where the interaction between pancreatic fluid and the hemostatic agents may be affected or changed in in-vivo environment. On the other hand, this insight offers a new perspective which can allow to the development of more potent agents in this setting.

CONCLUSION

In conclusion, Tisseel fibrin sealent has beneath its hemostatic properties also the potential of preventing pancreatic fistula development. Further in-vitro and in-vivo studies are needed to reach a definitive conclusion.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports

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