

# **Clinical Impact and Biomaterial Evaluation of Novel Hemostatic Microporous Polysaccharide Hemispheres in Cardiac Surgery**

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## **INTRODUCTION**

Perioperative bleeding leads to increased operating room time, blood product transfusions, pulmonary hypertension, and potentially to mortality. The coagulopathy induced by cardiopulmonary bypass and the multiple high pressure anastomoses created during cardiac surgery often result in bleeding, which is more effectively controlled with topical hemostatic agents than with sutures or electrocoagulation. Several topical hemostatic agents are available which either provides clotting components (e.g. fibrin sealants and thrombin glues) or a surface for clotting to be stimulated (e.g. microfibrillar collagen, gelatin sponge, oxidized cellulose). These agents have varying degrees of efficacy, some carrying a potential risk of infection or allergic reaction, adhesion formation, prolonged preparation time and considerable expense.

A new microporous polysaccharide hemisphere (MPH) product is a natural composition synthesized from a purified plant polymer and free of all biological components. When applied directly to an actively bleeding wound, each particle acts as a molecular sieve to instantly extract fluids from blood. This powerful osmotic action causes the particle to swell and concentrates serum proteins, platelets and other formed elements on its surface. The particles and their coating of compacted cells create a scaffold for the formation of a tenacious fibrin clot within just minutes of application. The particles are fully absorbed and enzymatically cleared from the wound site within 24 to 48 hours.

We conducted a randomized comparative clinical trial to compare the efficacy and safety of two standard clinical regimens of each topical hemostatic group vs. MPH.

- Fibrin sealant (Tisseel, Baxter-Eczacibasi, Istanbul, Turkey) consists of a two-component fibrin biomatrix that offers highly concentrated human fibrinogen to seal tissue and stop diffuse bleeding
- Gelatine (Microval-G.R.F. ®, Saint-Just-Malmont, France) glue consists of an aqueous mixture of gelatine and resorcine. The polymerization occurs by the addition of a hardener made of formaldehyde and glutaraldehyde.
- MPH (Arista™, Medafor Inc., Minneapolis, MN)

We have also collected aortic punch biopsy from each patient enrolled in the study, harvested endothelial cell culture and evaluated topical agents on biomaterial aspect with respect to cytotoxicity and foreign body reaction, adverse effect on wound healing, inflammatory reaction-resistance to infection and resorption-clearance time from the tissue.

## **PATIENTS & METHODS**

### **A) Clinical**

In a prospective, randomized, controlled trial, 90 patients (N=30 for each group) undergoing aortic valve surgery were enrolled. The clinical trial involving human subjects was conducted in accordance with the Helsinki Declaration, as amended by the 41st World Medical Assembly in Hong Kong in 1989, and US Code of Federal Regulations. The Institutional Review Board approved the study before patient enrollment. Before the operation, each patient signed an Institutional Review Board–approved consent form to participate in the study.

Patients were excluded if they were pregnant or if they had a known sensitivity to any components of bovine thrombin preparations or to any material of bovine origin. Patients were enrolled in the study intraoperatively if they did not have an active infection at the operative site, if there was bleeding that required the use of a topical hemostat, and if the use of a topical hemostatic agent was not contraindicated.

A baseline blood sample was obtained within 24 hours before operation to measure complete blood count, blood cell differentials, activated partial thromboplastin time, prothrombin time, and metabolic, hepatic, and renal panels.

Enrollment was limited to those patients in whom the surgeon was able to identify a bleeding site for which conventional means to stop bleeding (including direct pressure, suture, and electrocoagulation) were impractical or proved unsuccessful. After identification of a bleeding lesion requiring a topical hemostatic agent, the patients were randomized to fibrin sealant, gelatine and MPH. All bleeding sites in any patient were treated with the hemostatic agent to which the patient was randomized. The bleeding severity at each site was characterized as "oozing" or "heavy bleeding" (flowing or spurting). After application of the hemostatic product, the occurrence of continued bleeding was recorded at 1, 2, 3, 6, and 10 minutes. Reapplication of the assigned product was allowed, and the primary endpoint was cessation of bleeding of the first treated site within 10 minutes. Secondary endpoints included the outcome of additional treated bleeding sites and time to cessation of bleeding.

After use of either product, surgeons were queried about handling characteristics. The three questions asked addressed ease of application, ability of material to conform to tissue surfaces, and access to difficult-to-reach locations. The answers were graded on a scale of 1 (easy or well) to 5 (difficult or poor).

Safety and adverse effects were assessed at 12 to 36 hours and 6 to 8 weeks postoperatively. Adverse events were categorized as mild, moderate or severe and were assessed by the surgeons as unrelated, possibly related, or probably related to the products used. Hematologic and blood chemistry assays were performed as before at the evaluations 12 to 36 hours and 6 to 8 weeks postoperatively.

Effectiveness results were statistically analyzed in an “intent-to-treat” fashion using the Cochran-Mantel-Haenszel test stratified by site. The product handling questionnaire results were compared in a similar fashion. Comparison of the times to bleeding cessation was performed using the Gehan-Wilcoxon test.

## **B) Biomaterial**

Aortic punch biopsy from each patient was harvested in cell culture media. The quantity of cells and features were counted and verified by CD34 stain.

Topical agents were tested according to their

- cytotoxicity, foreign body reaction
- adverse effect on wound healing
- inflammatory reaction-resistance to infection
- resorption-clearance time from the tissue.

### **i) Cytotoxicity:**

Cytotoxic effects of fibrin sealant, gelatine and MPH were studied on cultivated human aortic wall endothelial cells (CE). CEs were passed in insert wells that allowed the apical side of CE monolayer in contact with topical agents. MTS proliferation bioassay and calcein-acetoxymethyl ester (CAM)-ethidium homodimer staining were performed to evaluate cell viability after CEs were co-cultured with agents for 48 h. Apoptosis of CEs was evaluated by TdT-mediated dUTP nick-end labeling (TUNEL) stain.

### **ii) Adverse Effects on Wound Healing:**

Cultured cell quantity was measured after 48 h until the end of one week after being in contact with topical agents. The increase or decrease in cell number was documented.

### **iii) Inflammatory reaction:**

Phagocytic capacity of cultivated endothelial cells was compared before and after contact with topical agents.

### **iv) Resorption:**

The cell cultures were observed by iodine testing and electron microscopy until two weeks after being in contact with topical agents checking for the clearance of agents from the cell clusters.

## **RESULTS**

### **A) Clinical**

A total of 90 aortic surgery patients (N=30 for each group) were screened for this study. The patients in fibrin sealant group had a total of 88 bleeding sites, gelatin group 85 and MPH patients had a total of 91 bleeding sites that were treated.

For both the first bleeding site treated, and for all the bleeding sites treated, the times to hemostasis were significantly shorter for the fibrin sealant and MPH groups compared with the gelatin group ( $p < 0.05$ ; Gehan-Wilcoxon test). At each time point, the percentage of successes in fibrin sealant and MPH groups was greater than the percentage of successes in the gelatin group ( $p < 0.05$ ; Gehan-Wilcoxon test).

The distribution of severity of bleeding classified as "oozing," or "heavy bleeding" was similar between the three groups. Data stratified by the severity of bleeding also showed fibrin sealant and MPH to be faster than the gelatin in stopping the bleeding. Hemostasis success (cessation of bleeding within 10 minutes) for the "oozing" category was 94% in the fibrin sealant, 91% in MPH and 66% in gelatin group ( $p < 0.05$ ). For the "heavy bleeding" category, the success rates were 92% in fibrin sealant, 89% in MPH and 40% in gelatin group ( $p < 0.01$ ). Fibrin sealant and MPH stopped bleeding statistically significantly faster than the gelatin in both the "oozing" group ( $p < 0.01$ , Gehan-Wilcoxon test) and the "heavy bleeding" group ( $p < 0.05$ ).

The responses to the question relating to the ease of applying product to the bleeding site were similar for both MPH and gelatin but significantly less for fibrin sealant ( $p < 0.05$ ).

Comparison of blood assays between baseline, 12 to 36 hours, and 6 to 8 weeks postoperatively, demonstrated no statistically significant differences that were judged to be clinically significant. No allergic reactions occurred in any groups.

## **B) Biomaterial**

The MTS bioassay showed that contact of gelatine and fibrin sealant inhibited CE proliferation. The suppressed cell cycling were significantly more in fibrin sealant and gelatine than MPH ( $p < 0.05$ ). CAM-ethidium homodimer staining revealed CE death,  $9.1 \pm 0.1\%$  in MPH vs.  $21.3 \pm 0.2\%$  in fibrin sealant and  $28.6 \pm 0.4\%$  in gelatine ( $p < 0.05$ ), but apoptosis played only minor role in groups

Phagocytic capacity was similar in three groups.

Resorption of agents from cultured media was  $52 \pm 11$  h for MPH,  $4.2 \pm 1$  days for fibrin sealant and  $11.2 \pm 3$  days for gelatine ( $p < 0.001$ ).

## **CONCLUSION**

MPH (Arista™, Medafor Inc., Minneapolis, MN) and fibrin sealant (Tisseel, Baxter-Eczacibasi, and Istanbul, Turkey) were statistically better than gelatine (Microval-G.R.F.®, Saint-Just-Malmont, France) with respect to faster hemostasis and hemostatic success rate. MPH and gelatin were easier to use. MPH was significantly better than two other groups with respect to biomaterial evaluation of adverse effects on wound healing and resorption rate.

MPH is a safe and effective topical agent for use as an adjunct to hemostasis in patients undergoing cardiac surgery.