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Antibacterial Activity of Some Commonly Used Hemostatic Agents in Maxillofacial Surgery

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After having been approved over the last few years, a variety of topical hemostatic agents are now commonly used in surgery. Hemostatic agents are used in oral and maxillofacial surgery in order to control bleeding; they can influence a surgical wound as a result of both their physical or chemical properties. The choice of any one of these agents depends varies according to a number of factors, including the surgeon's preference and experience. The aim of the study described here was to evaluate and compare the antibacterial activity of the following hemostatic agents: absorbable gelatin, aluminum chloride, oxidized cellulose, thrombin andferric sulfate), all of which are used in oral and maxillofacial surgery to control bleeding. The antibacterial effect of the five hemostatic agents was tested against five bacteria namely: *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus mutans*, *Streptococcus salivaris* and *Enterococcus faecalis.* TheBrain Heart Infusion agar well diffusion assay test was used to examine the antibacterial activity of the individual hemostatic agents. After incubation, the agar plates were examined for inhibition zones, which when present were measured in millimeters. When antibacterial activity was observed, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the tested agents werealso determined. Three of the tested hemostatic agents (Surgicel, Viscostst and Hemox A) showed antibacterial activity against all tested organisms. Absorbable gelatin and TachoSil however, did not inhibit the growth of any of the tested bacteria. The largest inhibition zones were produced by Hemox A, while Surgicel showed the smallest inhibition zones which ranged from 20-22 mm. Differences in the size of inhibition zones produced by Surgicel, Viscostat and Hemox A were statistically significant. The minimum inhibitory and bactericidal concentrations also varied between the three effective hemostatic agents. Hemox A was the most effective agent against tested bacteria followed by Viscostat and Surgicel. Since site infections continue to be a risk of surgical failure, the antibacterial properties of hemostatic agent should be considered when selecting such materials for the control of bleeding in maxillofacial surgery.

Key words: Antibacterial activity, Hemostatic agents, infection, Maxillofacial

Hemostatic agents are used in oral and maxillofacial surgery in order to control bleeding and as adjuncts to other methods of controlling hemostasis such as electrosurgery. Complications related to the use of hemostatic agents are caused by their chemical composition, location of placement and their absorption time(Palm and Altman, 2008). The application of any material to a surgical wound can affect the wound depending on its physical or chemical properties, while chemical compounds or foreign material can also influence the part played by bacteria in the infection of wounds(Jansen and Peters, 1993). A variety of topical hemostatic agents are now commonly used in surgery following their approval over the last few years. Many of these materials have varying degree of efficacy, but are generally very effective in controlling bleeding. The choice of such agents

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varies depending on a number of factors, including the surgeon'spreference and experience(Barnard and Millner, 2009). Some of these hemostatic agents are derived from human or animals and may cause reactions in the host.There is also the risk of cross infection andtransmission of viral agents when using such products. As a result, the operation may be a success only to be followed by a serious bacterial infection which then affect the total surgical outcome(Lotfi *et al*., 2008). Some hemostatic agent possess antibacterial activity (Dineen, 1976) and their careful selection and use can help reduce the risk of surgical site infections.

The aim of this study was to evaluate and compare the antibacterial activity of five different hemostatic agents (absorbable gelatin, aluminum chloride, oxidized cellulose, thrombin and ferric sulfate) used in oral and maxillofacial surgery to control bleeding.

MATERIALS AND METHODS

The antibacterial activity of the following hemostatic agents was determined: absorbable gelatin (Cutanplast, Italy), 25% aluminum chloride 6-hydrate (Hemox A, Deepak, USA), oxidized cellulose (Surgicel, Johnson and Johnson), human fibrinogen-thrombin (TachoSil, Switzerland), and ferric sulfate (Viscostat, USA). Brain heart infusion (BHI) agar wells diffusion assay test was used to determinethe antibacterial activity of different hemostatic agents. A bacterialculture (200 µl) was spread on the surface of BHI agar plates; five different bacteria were used in the antibacterial assay, namely: *Staphylococcus aureus*, *Staphylococcus epidermidis* (obtained from the College of Science, King Saud University), *Streptococcus mutans*, *Streptococcus salivaris*, *Enterococcus faecalis* (obtained from the Caries Research Chair Laboratory, College of Dentistry, King Saud University).Three uniform 4 mm diameter wells were then cut from the agar into which the solutions of the hemostatic agents were transferred. Non-liquid materials were suspended into distilled water for two days allowing the material to dissolve and 100 µl was the transferred to the wells. All plates were incubated for 24 hours at 37º C, and then examined for the presence of inhibition zones; any zones were then were measured in millimeters. When an agent showed antibacterial activity its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the individual bacteria was determined.

RESULTS

Three of the tested hemostatic agents (Surgicel, Viscostst and Hemox A) showed antibacterial activity against all tested organisms, while absorbable gelatin and TachoSil did not inhibit the growth of any of the bacteria. The largest inhibition zones were produced by Hemox A, with an average size of 39 mm. Viscostat inhibited the growth of all tested organismswith inhibition zones ranging from 28 to 30 mm for *S. aureus*, *S. epidermidis*, *S. mutans*, *E. faecalis* and *S. salivaris*. Surgicel produced the smallest inhibition zones, ranging between 20-22 mm (Table 1).The differences in the size of zones between Surgicel, Viscostat and Hemox A wasstatistically significant (p= 0.001). The minimum inhibitory concentrations for Hemox A was 11.8 µl/ml for *S. epidermidis* and *faecalis*, 24 µl/ml for *S. aureus*

Table 1. The antibacterial activity of the hemostatic agents measured using the agar diffusion assay

Bacteria	Hemostatic agent (100µl) Inhibition zone (mm)				
	Surgicel	Viscostat	Hemox A	Tachosil	Cutanplast
S. aureus	22 ±1	29 ±1	39.6 ±0.5	0	0
S. epidermidis	22.8 ±0.2	30 ±1	39.6 ±1.1	0	0
E. faecalis	20.3 ±0.5	29 ±1	42 ±1.7	0	0
S. mutans	22 ±1	31.3 ±0.5	39.6 ±0.5	0	0
S. salivaris	20.6 ±0.5	28 ±0.0	38.8 ±0.2	0	0

and *S. mutans* and 25 µl/ml for *S. salivaris.* Viscostat minimum inhibitory concentrations ranged from 24 to 50 µl/ml and for Surgicel, from 32 to 65 µl/ml (Table 2). The minimum bactericidal concentration for Hemox A for all bacteria ranged from 24 to 50µl/ml, while the maximum concentration of Surgicel to kill *S.aureus*was 85 µl/ml, while only 30 µl/ml was needed to kill *E. faecalis*.The MBC for Viscoststwas 64.3 µl/ml for *S. aureus* and 50- 50.6 µl/ml for the other bacteria.

Table 2. Minimum inhibitory concentration (MIC) of the hemostatic agents

Bacteria	Hemostatic agent (µl/ml)		
	Surgicel	Viscostat	Hemox A
S. aureus	65.6 ±1.1	50.3 ±1.5	24.6 ±0.5
S. epidermidis	32.3 ±0.5	24 ±1.0	11.8 ±0.7
E. faecalis	32.5 ±0.5	25 ±1.0	11.8 ±0.5
S. mutans	41 ±1.0	30.6 ±1.1	24 ±1.0
S. salivaris	45.3 ±0.5	34.6 ±1.5	25 ±0.5

Table 3. Minimum bactericidal concentration (MBC) of the hemostatic agents

Bacteria	Hemostatic agent (µl/ml)		
	Surgicel	Viscostat	Hemox A
S. aureus	85 ±1.0	64.3 ±0.5	50 ±0.5
S. epidermidis	65.3 ±0.5	50 ±1.5	25 ±0.4
E. faecalis	31.6 ±0.5	50 ±1.0	25 ±1.5
S. mutans	64 ±1.7	50.6 ±1.0	24 ±0.5
S. salivaris	64.3 ±0.5	50±1.0	50 ±0.5

DISCUSSION

Few studies have discussed the antibacterial properties of the hemostatic agents commonly used in maxillofacial surgery. Selection of the topical hemostatic agent is mainly governed by a surgeon's preferences and rarely by evidence based information. An investigation of the effect of five different commonly used hemostatic agents was carried out in this study. The effect was tested on microorganisms isolated from the oral cavity and from skin. The agar diffusion assay is often used to evaluate the antimicrobial properties of materials. The effect of the tested material depends on the degree of diffusion of its components across the medium. The results show that all three of the tested hemostatic materials exhibited antibacterial activity, with the degree of inhibition varying significantly between species and with Hemox A(which consists of aluminum chloride) showing the most marked inhibitory effect, followed by Viscostat and Surgicel. The mechanism of action of aluminum chloride in hemostasis is reported due to its hydrolysis to hydrogen chloride, which causes coagulation and vasoconstriction of the tissue in the immediate area of use. This material is caustic and its excessive application to the tissue may delay wound healing,although other studies have shown that aluminumchloride has antibacterial activity (Welage *et al*., 1994; Holzle and Neubert, 1982). A range of mechanisms by which aluminum might affect microorganisms have been reported, including its ability to bind to the cell wall and cause impaired permeability. Another suggested mechanism is that it replaces divalent metal complexes in cells or cell membranes and that it also complexes with ATP, DNA, and phosphates, leading to phosphate deprivation and enzyme inactivation(Yaganza *et a*l., 2004; Avis *et a*l., 2009). Ferric sulfate (Viscostat) is thought to

occlude the blood vessels by protein precipitation and my cause hyperpigmentation of the tissue by the deposition of iron particles. Although the mechanism of action of Surgicel as a hemostatic agent is still unknown it is thought that it works as amesh for clot formation. Dinee (1976)showed that oxidized cellulose (Surgicel) possesses an antibacterial activity, and the clinical application of Surgicel has been reported to reduce infection at surgical sites (Alfieri *et a*l., 2011).The antibacterial effect of Surgicel, which has been also used as a surgical dressing (Uysal *et al*., 2006), could be due to its acidity (low pH). The other two agentsthat were examined in our study namely absorbable gelatin and Tachosil did not exhibit antibacterial activity, a finding which agrees with a previous study by Dineen (1976). Absorbable gelatin works mainly as mechanical mesh which facilitates clotting;Tachosil, which contains thrombin, is also considered to be a physiological clotting agent. The results of the present study show that the two last named agents do not inhibit bacteria.

Most previous reports have concentrated on the antibacterial effect of Surgicel, together with its hemostatic properties. However, other materials, which are more effective antibacterial agents as well asbeing ideal hemostatic agents, should also be considered. In the present study the antibacterial effect of Surgicel was shown to be less than that of the other tested materials, such as aluminum chloride and ferric sulfate; these agents however, tend to be more toxic and, following their excessive application, may cause undesirable effects on tissue; as a result, they should be used with caution.Finally, as site infections present a major risk for clinical failure, it is suggested that the antibacterial properties of hemostatic agents should be taken into account when selecting such materials for controlling bleeding in maxillofacialsurgery

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