"A clinical evaluation of the efficacy of a new hemostatic device for topical use in the surgical management of patients undergoing anticoagulant therapy".
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1. INTRODUCTION

Over the last few years there has been a considerable increase in the number of patients taking drugs that, in different ways, are able to reduce the risk of thrombotic events.

Individuals at risk of thrombosis include patients with prosthetic valves, those with atrial fibrillation or deep vein thrombosis. The drugs prescribed to these patients for long-term treatments can be grouped into two broad categories: anti-platelet agents and oral anticoagulants. Both these categories of drugs reach their objective by modifying the physiological coagulation process.

The intake of anti-platelet agents per se is not contra-indicated with a view to performing oral surgery, since the onset of severe hemorrhage is an event considered as extremely rare in the literature. Treatment with oral anticoagulants, instead, requires careful monitoring of INR, and it is likely that local haemostatic agents will be required.

In this paper we will consider the pharmacologically induced coagulation deficits in patients taking oral anticoagulants, anti-platelets or fibrinolytics; we will examine the clinical protocols suggested in the Literature to follow in order to perform safely even the simplest dental treatment.

The purpose of this paper, after a brief evaluation of operational protocols in the literature, is to highlight the effectiveness of a new drug formulation made in collaboration with the Faculty of Pharmacy, University "Federico II" for hemostasis control in post extraction care in patients taking anticoagulants.
2. HEMOSTASIS

Several routine dental procedures may cause the bleeding of the oral mucosa which, in most cases, can be controlled without difficulty and with minimal blood loss from the patient. In some individuals, however, the physiological processes that allow the organism to control bleeding (hemostasis) are altered, which may favor the onset of bleeding. Although there are numerous diseases that can affect these mechanisms, the most frequent cause of hemostasis alterations is taking medications for the prevention of thrombosis, that is of drugs that act on the platelet phase (anti-platelet) and clotting (anticoagulants) of the hemostatic process.

Hemostasis is a physiological defense process and in vivo opposes any bleeding caused by injury or rupture of a blood vessel. It is this physiological process that ensures the integrity of the blood stream. This process is based on the balance between two phenomena: the formation of the clot, which has the function of protecting from bleeding, and the dissolution of the clot, which protects against thrombotic events. This is a so-called ‘two-way process’.

Hemostasis consists of four basic stages: also called ‘vascular’ phase (primary hemostasis), ‘platelet’ phase, ‘coagulation’ phase (secondary hemostasis) and ‘fibrinolysis’.

2.1 PRIMARY HEMOSTASIS

During the vascular phase the damaged vessels attempt to minimize blood loss; they do so through a vascular vasoconstriction which, however, is not able to ensure permanent hemostasis unless dealing with capillary lesions. Even in case of more severe injuries, the vasoconstrictor phenomenon is of considerable importance; such stimulus is present also during the successive stages of the coagulation process. The secretion of the following substances will take place: thromboxane, EDRF, NO and prostacyclin. In the same phase, the platelets release serotonin and noradrenalin that have a
vasoconstrictor effect.

**The platelet phase** has a role of considerable importance within the process of blood clotting. Very broadly speaking, we can distinguish five main stages: ‘adhesion of the first platelets to the damaged area’, the platelets interact (Glic.Ib) with the collagen of the perivascular tissue facilitated by the presence of von Willebrand factor; ‘activation of platelets adherent to the damaged surface’; ‘release of chemical signals contained in activated platelets’; ‘activation cascade of other platelets’ stimulated by the previous step and finally ‘platelet aggregation’; the latter is a reversible process because platelets have a tendency to disperse and, if the phase of coagulation does not take place, there is a resumption of the bleeding. The platelet phase, therefore, while being very important in the haemostatic process, is not sufficient to obtain definitive haemostasis.

2.2 SECONDARY HEMOSTASIS

**The coagulation phase (or plasmatic)** is the most important step in the process of blood coagulation. Under normal conditions, it leads to the arrest of the permanent blood loss. The plasma phase is finalized to the transformation of fibrinogen (a glycoprotein present in the bloodstream) into a fibrin clot, a protein that, in cooperation with the platelets, occludes the damaged area. In the last fibrinolytic stage there is the presence of another protein, plasminogen, which when converted to plasmin, degrades the fibrin clot finally restoring the vessel integrity (restitutio ad integrum); fibrinolysis, therefore, although part of the haemostatic process, is still an anti-haemostatic component (63).

The coagulation cascade is accomplished via two mechanisms that converge on a common pathway (Fig. 1)
**Figure 1: The coagulative cascade**

EXTRINSIC MECHANISM: cell membranes contain the tissue thromboplastin, and further to a lesion, this factor appears in the plasma, where it causes activation of the proconvertin (factor VII). This factor acts directly on the Stuart factor (factor X), activating it. Also thrombin appears to have an enhancer effect on the extrinsic mechanism, accelerating the activation of proconvertin.

INTRINSIC MECHANISM: its triggering depends on the plasma contact with negatively charged substances such as collagen and proteins of the basement membrane of the endothelium. This
determines the activation of the Hageman factor (factor XII) in a process which also involves two other factors: kininogen and prekallikrein. Factor XII activates factor XI (plasma thromboplastin factor), which in turn activates the Christmas factor (factor IX). Factor IXa, together with platelets, Ca ++ and phospholipids, cooperates with the anti-hemophilic globulin (factor VIII) for the activation of the Stuart factor.

COMMON PATHWAY: both previous processes converge in the common pathway, beginning with the activation of factor X; factor Xa 9 begins the conversion of prothrombin into thrombin, and this causes a positive feedback activation: thrombin activates proaccelerin (factor V), which binds to factor Xa to form the so-called "prothrombin activator"; while thrombin negatively regulates its activation by activating two plasma proteins (C and D) that inhibit proaccelerin and antihemophilic globulin.

Thrombin cuts the fibrinogen into insoluble fibrin; the stabilization of the fibrin (ie promoting the formation of covalent bonds between fibrin monomers) is made possible by a stabilizing factor (Factor XIII), previously activated by thrombin. Coagulation occurs rapidly, since each enzyme molecule activates several enzyme molecules of the next stage, amplifying the whole process. The final product is the coagulation cap (red clot), in which the fibrin constitutes the fibrous network of a gelatinous mass in which we find all the blood cells and a liquid part formed by the plasma⁶².

CLOT RETRACTION: Once formed, the clot decreases its mass; there is, in fact, the draining of serum (ie the plasma deprived of the fibrinogen). The retraction is used to promote the adhesion of the clot to the injured tissues.

FIBRINOLYSIS: the dissolution of the clot occurs thanks to the demolition of fibrin by plasmin, derived from inactive plasminogen. In addition, plasmin is capable of degrading important X factors of coagulation 10: fibrinogen, factors VIII, V, XII. Multiple factors are capable of converting
plasminogen into plasmin. The main one is the tissue plasminogen activating factor, released from damaged tissues. Even some factors of the intrinsic coagulation mechanism are capable of converting plasminogen into plasmin. This is a clear confirmation of the fact that the coagulation and fibrinolytic process are in dynamic equilibrium.

2.3 WOUND HEALING

The healing following a wound begins with the formation of a clot consisting of fibrin and platelets.

This clot has the immediate effect of blocking the blood loss, and so as a result, the fibrin and the growth factors contained in the platelets allow the repair of new tissue.

Fibrin is rapidly invaded by leukocytes which are the first cells to start the neo-vasculature; the white blood cells contain VEGF, the most potent among the vascular endothelial growth factors. The platelet growth factors (PDGF) are equally involved in neo-vasculature and fibrin serves as the tissue matrix to allow the reconstruction. Healing can occur by primary or secondary intention.

*Healing by primary intention (surgical wound):* the loss of substance caused by surgical incision is filled by a blood clot consisting of a network of fibrin which contains red blood cells, white blood cells, platelets and other blood components. In this phase, the clot, very adherent to the walls, can also be easily removed by small traumas. In the following period the macrophages appear. They are mononuclear cells with phagocytic capacity, and therefore active in cleaning up the wound from fibrin and cellular debris.

After the first 24-48 hours a granulation tissue is formed consisting of some mobile elements that originates from the connective fibroblasts. They penetrate into the wound along the filaments that make up the network of fibrin, replacing them with fibers with high capacity contractile myofibrils.
Simultaneously, on the margins of the wound begins the production of vascular and later lymphatic formations that extend gradually towards the center until they meet with the same vascular formations on the opposite side. Once the anastomosis of the stumps has taken place a channeling process starts by which cellular cords become vessels and form a new vascular network. Fibroblasts also have the ability to secrete a substance, hyaluronic acid, the active component in the formation of collagen fibers; in this phase the wound appears swollen and reddened for the richness of newly formed vascular tissue.

With the passing of time the number and activity of fibroblasts decreases, blood capillaries are reduced and simultaneously the number of collagen fibers increases. The transformation of the granulation tissue into scar tissue takes place; its characteristics are low elasticity, reduced innervations and blood supply, modest epithelialization, the absence of skin appendages.

This process leads to the formation of a solid scar in about two weeks. During this time it becomes gradually contracted because of the action of the myofibrils.

*Healing by secondary intention:* this healing is characterized by a significant loss of tissue which leads to a more intense inflammatory reaction and the formation of larger amounts of granulation tissue which, starting from the bottom of the wound progresses, very slowly, upwards. It is a longer and more tedious process than primary healing, often responsible for serious imperfections. The grainy look, which gives it its name, becomes evident and its rich vasculature may easily lead to bleeding.
3. MANAGEMENT OF PATIENTS AFFECTED BY COAGULOPATHY

The management of patients receiving anti-platelet agents and anticoagulants, as they suffer from conditions at high risk of thrombosis, is a big commitment. For these patients wound healing is a slow and difficult process for the interference of anticoagulant drugs with the mechanisms of formation of the clot (responsible for immediate hemostasis).

In addition, cardiovascular patients often take, in combination with anticoagulants and anti-platelet therapies, also medications that can cause oral conditions such as: dry mouth syndrome and lichenoid reactions (β- blockers and anti-hypertensives), hypertrophic hyperplastic gingivitis (Ca - antagonists such as nifedipine), recurrent oral ulcers (anti-hypertensives as alpha-methyldopa); which is why the patient should be assessed as a whole.

These patients may be suffering from the following diseases: chronic stable angina, polycythemia vera, unstable angina, acute myocardial infarction, transient ischemic attack, ischemic stroke, severe carotid artery stenosis, atrial fibrillation (Table I).
Table I. Anti-Coagulant Therapeutic Indications

<table>
<thead>
<tr>
<th>Heart Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Atrial Fibrillation</td>
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<tr>
<td>- Prosthetic valve</td>
</tr>
<tr>
<td>- post- infarction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trauma surgery pathologies and vascular diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Peripheral arterial Disease</td>
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<tr>
<td>- Deep Vein Thrombosis</td>
</tr>
<tr>
<td>- Pulmonary Embolism</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Reconstructive Vascular Surgery</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Congenital or acquired pathologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Reduced sensitivity to activated protein C</td>
</tr>
<tr>
<td>- Deficiency of physiological blood clotting factors</td>
</tr>
<tr>
<td>- Fibrinolysis alteration</td>
</tr>
<tr>
<td>- thrombocytosis</td>
</tr>
<tr>
<td>- Blood hyperviscosity</td>
</tr>
</tbody>
</table>
The behavior of the dentist has to adjust to the different kind of treatment the patient is undergoing. If a patient is following an anti-platelet therapy, the monitoring of the therapy is not needed. Actually, in patients following an anti-platelet therapy, the monitoring of the bleeding time or of any other types of assessment is not necessary immediately before the intervention, since the anti-platelet therapy, unlike the anticoagulant one, is not subject to major variations over time and it is rare that in such cases a patient reaches high bleeding times. In fact, in patients following an anti-platelet therapy with low-dose aspirin, the risk of bleeding is very low and it occurs only in very rare cases when the bleeding time exceeds 20 minutes.

In the past, the risk of excessive bleeding led clinicians to discontinue anti-platelet therapy with low-dose aspirin before performing surgery, even if doing so exposed the patient to the risk of a thrombotic event\(^{(44,45)}\).

Today such behavior is no longer shared on the basis of numerous reviews in the literature, which suggest that the majority of surgical procedures in the oral cavity can be implemented successfully without suspending the anti-platelet therapy with low-dose aspirin. A study of 51 patients on chronic anti-platelet therapy who underwent surgery in the oral cavity without discontinuation of the therapy, has recorded a single case of excessive intra-operative bleeding and no cases of postoperative bleeding. The only bleeding episode occurred after the extraction of a third molar and hemostasis was in any case obtained through stitching and the application of gauze soaked in tranexamic acid\(^{(46)}\).

In conclusion, we can state that there is no need to suspend or change the chronic anti-platelet therapy before performing surgery to the oral cavity. In the rare cases of a patient requiring urgent surgery and bleeding time exceeding 20 minutes, desmopressin or any other similar medication can be administered in order to reduce bleeding time\(^{(47)}\).
3.1 HEMATOCHEMICAL EVALUATION PARAMETERS

The therapeutic use of oral anticoagulants, especially if prolonged over time, requires, instead, an extremely accurate monitoring. The measurement of the anticoagulant response, carried out by regular laboratory controls, allows to ascertain the presence of an anticoagulant effect for the duration of the treatment and to maintain the coagulation levels within the recommended range for the pathology to be treated.

The values of such a range were derived from longitudinal studies of numerous patients at risk of thrombosis, which allowed to obtain these minimum and maximum statistical values able to reduce thrombotic phenomena without causing spontaneous hemorrhage.

The required dosage of the drug to reach the individual therapeutic range varies from subject to subject.

This complicates the treatment protocol and for this reason the attempts to reach a stable therapeutic range are often as long as a few months. Once the goal of a stable range has been reached, the coagulation checks are carried out only every 15-20 days.

The anticoagulation control is always performed by means of a test introduced by Quick in 1935, which explores the extrinsic pathway of the coagulation as it is sensitive to a reduction of some vitamin K dependent coagulation factors (II, VII and X) inhibited by oral anticoagulants. The test measures the time it takes for the blood to coagulate following the addition of an optimum amount of tissue thromboplastin and calcium ion. The tissue thromboplastin interacts with the various vitamin K dependent factors and determines the formation of thrombin from prothrombin. A prolonged clotting time indicates the inhibition of such coagulation factors.

Following a number of drawbacks occurred over the years linked to the use of the PT, probably caused by the progressive deterioration of thromboplastin used as a reagent, numerous attempts have been
made in order to standardize the various techniques used in the evaluation of the activity of oral anticoagulants.

In 1976 the "WHO has indicated as a primary reference thromboplastin a substance called 67:40 by the International Committee on Thrombosis and Haemostasis (ICTA), ie a thromboplastin derived from human brain. The limited availability and the impossibility of sending the reagent in all parts of the world have pushed the ICTA to propose that the European Community Bureau of Reference undertake a calibration study of thromboplastin internationally. Comparing a thromboplastin to another is difficult for a whole series of biochemical and methodological reasons. These difficulties have been avoided in part, within the therapeutic range of anticoagulants, by introducing a linear relationship of the logarithms of prothrombin time, calculated by performing an orthogonal regression equation, and taking the gradient as the relation index. It was proposed that the gradient of the correlation between any thromboplastin and the primary international reference preparation should be called "International Sensitivity Index" (ISI) in accordance with the WHO recommendations. The manufacturers were thus invited to introduce, as soon as possible, their own internal standard for the thromboplastin produced by them and to indicate by means of a label the gradient of the batch of the material with respect to the international reference thromboplastin.

It was then introduced a new prothrombin index as an expression of the time defined INR (International Normalized Ratio). The INR is therefore the prothrombin ratio that would be observed if the prothrombin used were the international reference material, derived from a standard preparation and filed with the WHO. The INR is equal to the ratio of the patient's prothrombin time raised to the power of the ISI value (International Sensitivity Index) for the analytical system used.

\[
INR = \left( \frac{PT_{\text{paziente}}}{PT_{\text{riferimento}}} \right)^{ISI} = (PT \text{ ratio})^{ISI}
\]
The normal value is INR 1, while higher values indicate a prolonged prothrombin time, which means difficulty in coagulation.

Currently the monitoring of the action of a given oral coagulant is based on the use of the INR \(^{(48)}\). The adoption of the INR has made it possible to have a unitary system for recommended therapeutic ranges (range) in various diseases (Table II) and, in effect, thanks to it polycentric clinical studies have been carried out including the comparison of data from different laboratories.

After the establishment of the therapy and the achievement of optimal coagulation levels, the time interval that elapses between the various controls of the INR can be gradually lengthened, consistently with the stability of the checks made, but the interval rarely exceeds 3 or 4 weeks, because of the many factors (diet, physical activity, fever, climate and drug interactions) that can interfere with anticoagulant activity. This monitoring is recorded, along with the administered doses, on a card that is updated at every check and delivered to the patient with the recommendation to produce it before undergoing any medical or surgical treatment.
Table II: INR suggested values in conditions requiring anti-coagulant therapy

<table>
<thead>
<tr>
<th>Clinical Situation</th>
<th>INR INTERVAL</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral commissurotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Intracavitary thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-rheumatic atrial fibrillation</td>
<td>2.0-3.0</td>
<td>3 months 0 sine die</td>
</tr>
<tr>
<td>Heart Biological Prosthetic valves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral valve stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myocardial Infarction</td>
<td>2.8 - 4.8</td>
<td>3 years</td>
</tr>
<tr>
<td>Heart Mechanical Prosthetic valves</td>
<td>3.0 - 4.5</td>
<td>Sine die</td>
</tr>
<tr>
<td>Deep Vein Thrombosis</td>
<td>2.0 - 3.0</td>
<td>3-6 Months 0 Sine die</td>
</tr>
<tr>
<td>Pulmonary Embolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral arterial Disease</td>
<td>2.0 - 4.0</td>
<td>Sine die</td>
</tr>
<tr>
<td>Reconstructive Vascular Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DVT Prevention in Surgery</td>
<td>2.0 - 2.5</td>
<td>Days 4-10</td>
</tr>
</tbody>
</table>
3.2 SUGGESTED PROTOCOLS FOR CLINICAL TREATMENT

Since the early 90s, patients on anticoagulants who had to undergo dental procedures associated with bleeding risk were made to discontinue the therapy or, in cases where this was not possible, the patient was hospitalized in order to ensure a better control of the coagulation before and after the operation \(^{(49)}\). The discontinuation of the anticoagulant therapy entailed an increased risk of thrombosis and imposed a difficult collaboration between the various specialists who had treated the patient. For these reasons, in later years, and following the introduction of INR monitoring method by the WHO (1983) the general attitude became not to reduce nor discontinue the anticoagulation therapy, but to keep it unchanged \(^{(50)}\); the works reported in the literature are directed, on the one hand, to investigate the difference in incidence of thrombotic complications and / or bleeding in the patients whose therapy has been suspended from those that continue the therapy; on the other, to assess whether haemostatic agents are able to control postoperative bleeding. A 1999 study, conducted on 150 patients (359 extractions), reported bleeding complications in 11 patients with INR between 1.5 and 4.0. in all cases haemostasis was easily obtained with the aid of local haemostatic (gelatin sponge, fibrin glue, sutures) \(^{(51)}\). Other studies have confirmed a low number of bleeding complications in patients undergoing anticoagulant therapy in oral surgery. Martinowitz et al. \(^{(52)}\) in patients with INR range 2.5-4.0 observed no bleeding complications after 63 extractions. Even Bonder et al. \(^{(53)}\) after 69 extractions have not detected bleeding with INR greater than 2 in 49 out of 69 patients.

3.3 DENTAL DISORDERS IN PATIENTS UNDERGOING ANTI-COAGULANT/ANTI-AGGREGANT TREATMENTS

It must be emphasized that there are two issues to be considered more carefully when planning the treatment of a patient on anticoagulant therapy. They are:
• the type of dental treatment

• the INR values and the underlying disease (INR range) (8-18)

Type of dental treatment

Not all dental treatments expose the patients to the risk of bleeding, as not all cause bleeding. Among the dental treatments associated with bleeding risk the following should be considered: extractions, placement of implants, periodontal surgery and root planing, and minor oral surgery. A risk factor in dental surgery is the use of local anesthetics in the sites which require surgery.

INR values and underlying disease

In patients receiving anticoagulants, before proceeding we must properly assess the extent and effectiveness of the current therapy, that is, the INR range (clotting degree).

Entities of therapy (INR): Before starting surgery on a patient on anticoagulant therapy it is essential to have a value of INR referring to the previous days because of the extreme variability of the INR. The dentist has the duty to evaluate on the one hand if the INR is within the therapeutic range for the disease from which the patient is suffering, and on the other hand if this value is compatible with the planned dental treatment.

Effectiveness of therapy (underlying disease): the dentist who is going to treat a patient on anticoagulant therapy should be very familiar with the thromboembolic risk of the patient, that is, with the disease for which the therapy has been started and what INR range required by this disease. As described in Figure 2, the primary goal is to keep the patient still within its therapeutic range, ie maintaining a INR value that exposes him/her, at the same time, to the least thrombo-embolic risk and to the least risk of bleeding.
**Figure 2:** Thrombotic risk and the risk of bleeding during anticoagulant therapy. The therapeutic range for most conditions is between 2.0 and 3.0: by increasing the INR there is a reduction of thrombo embolic risk (downward arrow) and an increase in haemorrhagic stroke (upwards arrow) and vice versa. 
INR VALUES AND TYPE OF TREATMENT

• **INR <2.0**: it is possible to perform any dental procedure, including those at high risk of bleeding, without modifying or suspending the anticoagulant therapy. We must assess whether such a low INR value corresponds to the patient's therapeutic range, otherwise his/her cardiologist should be contacted.

• **INR> 4.0**: No dental procedure should be performed outside a hospital or specialized structure; for such high values of INR, a cardiologist should be contacted to alter anticoagulant management. Currently there are very few indications to maintain the INR> 4 and the risk of this value is often correlated to an incorrect control of the therapy; that is why dentists who experience a higher value than the patient's INR therapeutic range should immediately inform his/her cardiologist.

• **INR> 2.0 and <4.0**: this range includes the majority of patients on anticoagulant therapy. We must assess the complexity of the surgery from time to time and correlate the local bleeding risk (type of surgery, duration, location) with the cardiologic risk (severity of heart disease), and with the thromboembolic event that would result from a change in the therapy. In general, in these cases it is indicated not to suspend the anticoagulant therapy so as to not increase the risk of thromboembolism but to control the bleeding through the use of tranexamic acid or other hemostasis adjuvants before, during and after surgery. Also, since the anticoagulant effect remains for several days after the discontinuation of the therapy, the practice of suspending anticoagulant therapy in the days preceding the intervention is not accepted today as this would increase the risk of thromboembolism without reducing the risk of bleeding. The change of the coagulation capacity is closely connected to type of treatment undergone by the patient. The careful examination of various types of drugs will be the subject of the next chapter.
4. ANTITHROMBOTIC MEDICATIONS

4.1 ANTI-AGGREGANTS

ASPIRIN

Acetylsalicylic acid (ASA) is a salicylate ester of acetic acid in which the carboxyl group of salicylic acid is substituted in correspondence of the hydroxyl. This compound was introduced into medicine in 1899, under the name of aspirin. It possesses a wide range of pharmacological effects which depend on numerous variables, including the dosage employed. In fact, while the anti-platelet effect is already present at reduced doses of 0.5-1 mg / kg, the analgesic and antipyretic ones require at least 5-10 mg / kg and the anti-inflammatory effect can only be appreciated in daily doses greater than 30 mg / kg (37).

Aspirin induces a long-lasting functional deficiency of platelets, clinically recognizable by the prolongation of bleeding time. This effect seems to be almost exclusively related to the activation of a permanent limiting enzyme platelet metabolism of the arachidonic acid. This enzyme is known as prostaglandin H (PGH) synthase and is responsible for the generation of PGH2. The latter is a substrate for some isomerase that generate at least five different bioactive prostanoids such as thromboxane A2 (TXA2) and prostacyclin (PGI2). Due to its activity of oxygenase (COX), the PGH synthase cycle is also defined and COX exists in two forms: COX-1 and COX-2. The low-dose aspirin selectively inhibits COX-1, while high doses of aspirin inhibit both COX-1 and COX-2.

These differences are probably responsible, at least in part, for the necessity of using very high doses of aspirin to obtain the analgesic and anti-inflammatory effects, while the anti-platelet effect can be obtained also with the daily dosage of 30 mg (38).

Since the inactivation of COX by aspirin is irreversible, a synthesis of the enzyme is required again to restore normal cell function that occurs within a few hours in the nucleated cells, but not in platelets.
that are derived from the cytoplasmic fragmentation of megakaryocytes, so they can synthesize only small quantities. Therefore the duration of platelet and extra-platelet effects of a single dose of the drug varies greatly and is between several days and a few hours, respectively \(^{(39)}\).

Aspirin seems to have other effects on haemostasis, beyond that produced by the inhibition of fibrinolysis and the suppression of coagulation \(^{(40)}\).

Despite the rapid clearance of aspirin, its anti-platelet effect has the duration of platelets’ life due to the permanent activation of COX; as a consequence, the effect of aspirin regresses only with the formation of new platelets. There is therefore a complete separation between the pharmacokinetic and pharmacodynamic effects of aspirin, which provides the possibility to administer it once a day to obtain the chronic anti-platelet action despite its short half-life \(^{(38)}\).

Randomized clinical studies indicate that low-dose aspirin may prevent arterial thrombosis both in the case of primary vascular events in healthy or low-risk patients (primary prevention), and in the case of recurrences in patients with known chronic or acute vascular occlusive disease (secondary prevention) \(^{(40)}\). The low-dose aspirin effects: in the newly formed human platelets COX-1 and COX-2 are present, while mature platelets only express COX-1. On the other end, vascular endothelial cells express both COX-1 and COX-2. The TXA2, which constitutes an amplification of platelet mechanism, is synthesized and secreted by platelets in response to different stimuli (collagen, thrombin, adenosine diphosphate) and, in turn, induces irreversible platelet aggregation \(^{(41)}\).

On the contrary, PGI2 inhibits platelet aggregation. The reduced formation of prostanoids (TAX2, PGI2) in different tissues can probably explain the wide range of the pharmacological effects of aspirin, which constitute the basis both of its therapeutic use and of its toxicity. The TAX2 derives primarily from the action of COX-1 and its synthesis is therefore highly reduced by the action of aspirin, instead
PGI2 derives mainly from COX-2 and, consequently, its synthesis is not changed by low doses of aspirin\(^{38}\). In conclusion, although other mechanisms have been proposed, the inhibition of platelet COX-1 is sufficient to explain the antithrombotic effect of aspirin at low doses.

**DIPYRIDAMOLE (PERSANTIN)**

Dipyridamole is a vasodilator which in combination with warfarin inhibits the embolization of cardiac-valve prostheses; when used alone it has modest antithrombotic effects.

Dipyridamole interferes with platelet function increasing the cellular concentration of cAMP. Today the only recommended use is in combination with warfarin for the primary post-surgery prophylaxis of thromboembolism in patients with prosthetic heart valves.

**TICLOPIDINE (TICLID)**

Ticlopidine belongs to the class of anti-platelet agents, and is used in all those diseases where there is a state of hypercoagulable blood that can lead to serious cardiovascular events (myocardial infarction, transient ischemia, cerebral stroke).

Ticlopidine is a pro drug that is converted to an active thiol metabolite which binds permanently to the platelet receptor P2\(\text{Y}\)\(^{48}\) and inhibits it preventing the aggregation of thrombocytes. It possesses, like aspirin, a short half-life and long duration of action: the maximum inhibition of platelet aggregation becomes evident only after 8-11 days of treatment.

Before a therapeutic effect becomes evident, in fact, more days of therapy are needed and of course its suspension does not immediately recover the platelet function, because the recovery occurs only after a period of time necessary for the creation of new platelets. Among the side effects that have led to the dropping of the use of ticlopidine should be mentioned those of hematologic nature namely agranulocytosis, a serious event, or thrombotic thrombocytopenic purpura (TTP), which can occur two
or three weeks after therapy onset.

CLOPIDOGREL (PLAVIX)

It is an anti-platelet agent of the thienopyridine family. Its main use is in the treatment and secondary prevention of acute myocardial infarction and acute coronary syndromes. It can also be used in the treatment of cerebral ischemia and peripheral vascular occlusive disease. It is closely related to ticlopidine, but seems to have a more favorable toxicity profile with less neutropenia and leucopenia. It is a pro drug with slow onset of action, has a synergic effect with aspirin and the usual dose is 75 mg per day.

4.2 ANTICOAGULANTS

Anticoagulants, as their name implies, are drugs that hinder the process of blood coagulation. They can be divided into two groups:

• Injectable Heparin

• Dicumarolics, administered orally.

HEPARIN

Heparin is a glycosaminoglycan that is found in secretory granules of mast cells (mast cells). It is prepared starting from the cells of the pigs’ intestinal mucosa or from beef lung. It can be administered by intravenous infusion or subcutaneous injection. It is divided into high-molecular-weight heparin (standard) and low molecular weight (fractionated). The fractionated heparin is frequently used due to its easily predictable pharmaco-kinetic profile that allows to control the subcutaneous administration depending on the weight without laboratory monitoring. Heparin binds naturally to a blood factor, antithrombin III, an inhibitor enzyme that as a result of the bond with the same heparin changes
conformation exposing its active site. The activated AT-III in turn deactivates thrombin (factor IIa), factor X, and other proteases involved in blood clotting. AT-III binds specifically to a pentasaccharide sequence of heparin sulfate contained in the polymer sequence. GlcNAc / NS (6S) -GlcA-GlcNS (3S, 6S) - IdoA (2S) -GlcNS (6S)

It is the conformational change in AT-III as a result of the bond with the heparin that allows the deactivation of Factor Xa by antithrombin itself. This deactivation of the active X factor also requires a link between the heparin and pentasaccharide factor Xa itself. These interactions are possible thanks to the high electronegative charge density of heparin; the formation of a ternary complex between AT-III, thrombin and heparin leads therefore to the deactivation of thrombin itself. The formation of the ternary complex thus depends on the length of the polymer heparin (at least 18 disaccharide units). The inactivation of factor Xa, instead, requires only the pentasaccharide.

Heparin then catalyzes the inhibition of several coagulation proteases thanks to antithrombin, a polypeptide synthesized by the liver which inhibits coagulation factors activated by the intrinsic and common pathway.

Heparin is used as an initial treatment of venous thrombosis and pulmonary embolism, due to its fast action, for the initial control of patients with unstable angina or acute myocardial infarction, during and after coronary angioplasty or stent placement, during procedures requiring cardiopulmonary bypass and to prevent venous thromboembolism in high-risk patients after orthopedic surgery. Also it appears to be the drug of choice in pregnancy being unable to cross the B.E.E and not having been associated with birth defects.

FRACTIONATED HEPARINS

The new generation fractionated heparins, administered subcutaneously, are extremely easy to handle
drugs and their therapeutic effect can be programmed especially for their duration, so they do not require to be monitored for their routine use, as for example in dialysis patients. In patients treated with heparin, monitoring may be required only in cases of continuous infusion; in these cases the most suitable laboratory test is the partial thromboplastin time (PTT).

The question of the need to suspend the concurrent anticoagulant therapy of surgical procedures in the oral cavity has been debated for many years: the protocols and standards of conduct proposed for the management of anticoagulation therapy in patients who are receiving dental care are numerous and diverse.

WARFARIN

It is a coumarin anticoagulant vitamin K antagonist. Various coagulation factors (prothrombin and factors VII, IX and X) in order to become active should undergo post translational modifications which consist in the carboxylation of certain glutamic acid residues, in order to generate γ-carboxyglutamic acid. During the reaction of carboxylation, vitamin K, which fixes and then transfers the CO2 molecule, is converted to vitamin K epoxide which is then converted back to the previous form by means of a specific reductase. This enzyme is the target of the action of warfarin, which determines its inhibition.

In order to make the anticoagulant effects of the drug appear, it is necessary that the pool of vitamin K is largely converted into epoxide. Only then, in fact, the factors of coagulation products will not be made active and will not be able to exert their action. In addition, some coagulation factors have a half-life of a few days: it is necessary to wait for them to become naturally consummated or degraded to achieve complete pharmacological action. It is for these reasons that the effects of the drug begin to appear 8-12 hours after ingestion and reach maximum effect after 48-72 hours.
The usual dosage of warfarin in adults is 5 mg per day for 2-4 days, followed by 2-10 mg per day as indicated by the values of INR.

*Latency time of oral anticoagulants*

The effects of oral anticoagulants, in contrast to those of heparin, are not immediate, but there is a latency time which depends on the half life of coagulation factors present in the blood and therefore still active (its half-life can reach up to 60 hours for some factors), as well as individual phenomena related to the absorption and elimination of the drug, the liver and the concomitant use of other substances capable of counteracting or increasing the action of these drugs. The diversity of all these factors makes the response to the same dose of drug extremely subjective.

The factors with shorter half-life, such as factor VII, whose half-life is 6 hours, will disappear prematurely, leaving the place to inactive forms, whilst molecules such as prothrombin, factor IX and factor X which have half-lives of 60, 24 and 40 hours, respectively, will require longer times.

Since the beginning of anticoagulation, therefore, it takes several days before getting a full anticoagulant effect; Similarly, after stopping the drug, it takes as many days to return to normal clotting.

The question of the need to suspend the concurrent anticoagulant therapy of surgical procedures in the oral cavity has been the subject of debate for many years: the protocols and standards of conduct proposed for the management of anticoagulation therapy in patients who are receiving dental care are numerous and mutually different.
4.3 NEW GENERATION ORAL ANTICOAGULANTS

DABIGATRAN

The direct thrombin inhibitors act by modulating the transformation of fibrinogen into fibrin and inhibit the thrombin-mediated activation of factors V, VII, XI, XII with anticoagulant effect; the block of thrombin determines also an inhibition of its receptor-mediated effects or platelet aggregation. Dabigatran\(^{(60,63)}\), a potent reversible direct thrombin inhibitor, both in its free form and when bound to fibrin, inhibits the activity of thrombin but also its generation. Dabigatran produces predictable and consistent pharmacodynamic effects, therefore it does not require regular coagulation monitoring or dose adjustment. In the course of the treatment the level of anticoagulation can be evaluated by measuring the "thrombin clotting time" although it is not yet a standardized test, or the "ecarin clotting time" a test, however not easily available on a large scale; these assays may be useful in case of bleeding and in case of suspected overdose, conditions in which even the aPTT may be lengthened. Alongside the efficacy results and safety of the therapy with dabigatran further advantages are represented by the lower inter-individual variability of response, by the lower profile of drug interactions and by the fact that the routine monitoring of coagulation is not needed; disadvantages linked to the new therapy are traced to the lack of availability of a specific antidote of dabigatran, therefore in case of severe bleeding supportive therapy with blood transfusions and plasma transfusions are required; to the twice-daily dosing that may reduce the therapeutic compliance and the high cost.

As pointed out in the update of the American guidelines because of the twice-daily dosing and the highest risk of non-bleeding side effects associated with the inhibitor of thrombin, patients taking warfarin with excellent INR control would not derive many benefits from the therapeutic switch; as opposed to those with poorly controlled INR, requiring frequent dose adjustments or frequent monitoring of coagulation, and with high probability of drug interaction.
RIVAROXABAN-APIXABAN

Factor Xa is a tempting target for the design of new anticoagulant molecules; positioned at the beginning of the common pathway of the coagulation cascade its inhibition increasingly reduces the formation of thrombin upstream, but does not block the circulating thrombin whose traces can intervene in hemostasis giving this therapeutic strategy a greater safety profile regarding the risk of bleeding. Rivaroxaban has been approved for the prevention of venous thromboembolism in patients undergoing hip and knee replacement surgeries and its efficacy in atrial fibrillation has also been assessed. FDA regulations regarding the use of this molecule are awaited; currently the use of rivaroxaban has been suggested in patients with inadequate response or who cannot take warfarin or dabigatran.

4.4 LOCAL HEMOSTATICS

To control bleeding the dentist has numerous both systemic and topical pharmacological aids; the latter include:

- **Oxidized regenerated cellulose**: fibrous, fibril-structured, sterile, absorbable haemostatic plug achieved through the controlled oxidation of regenerated cellulose. Once saturated with blood, it swells and becomes a gelatinous mass that contributes to the formation of the clot. It is also a bactericidal agent against several strains; therefore, it does not increase the risk of infection \(^{(18,23)}\).

- **Absorbable gelatin**: consists of a sponge of haemostatic absorbable gelatin, insoluble in water, at a constant porosity, digested in pepsin and sterile; It is an implantable hemostat and like all haemostatic gelatin sponges is intended to facilitate the arrest of bleeding, not to be used on infected wounds; it may or may not be associated with tranexamic acid \(^{(20)}\).

- **Collagen**: invasive device intended for short-term use, it is manufactured utilizing animal tissue \(^{(9,30)}\).
- **Fibrin based glues**: They contain thrombin, pro-accelerin, calcium, factor XIII and antifibrinolytic substances that lead to the formation of fibrin locally and reduce fibrinolysis \(^{(15,18,20,21,22,23)}\).

- **Glues based on cyanoacrylates**: They consist of a synthetic surgical glue based on cyanoacrylate, when they come into contact with the wound, they polymerize rapidly creating an elastic film; they have an adhesion and haemostatic action.

- **PRP (Platelet-Rich Plasma)** is a derivative plasmatic autologous, obtained after 2 centrifugations from the patient's blood, obtained with the addition of calcium and bovine thrombin that activate the coagulation cascade; its preservation is possible for a few days at a -19 ° C temperature. Scientific studies (Galindo-Moreno et al. 2005) have confirmed and highlighted the presence of fibrillar and cellular components SEM and TEM (mainly platelets). This structure would be capable of being a vehicle for cells and growth factors (PDGF, TGF-BETA, IGF) indispensable for the regeneration of soft and hard tissues (Marx et al. 1998; Anitua et al. 1999). The disadvantage of the PRP is the modest risk of anti-thrombin antibody formation for the activation of PRP with heterologous thrombin and high amount of blood to be taken; there are also legal medical problems regarding the handling of biological material that cannot be performed by the dentist.

Among the haemostatic agents used systemically there are:

- **Synthetic antifibrinolytic Amino acids**: two synthetic amino acid lysine derivatives, epsilon-aminocaproic acid (EACA) and tranexamic acid, have high anti-fibrinolytic activity in humans \(^{(24,26,32)}\). Both drugs bind reversibly to plasminogen and thus block the binding of plasminogen to fibrin itself and its activation to plasmin. Their distribution in the extravascular space and their accumulation in tissues is at the basis of efficacy in hemorrhagic conditions caused by local hyper-fibrinolysis. The aminocaproic acid and tranexamic acid (which is about 10 times more potent and has a longer half-life)
are effective even when the bleeding is not associated with signs of hyperfibrinolysis laboratory. They can be administered both orally and intravenously; they are eliminated in active form in the kidney, they are concentrated in the urine (up to 100 times) and pass in other body fluids (CSF, synovial fluid and sperm).

• **Aprotinin** is a polypeptide with a molecular weight of 6512, extracted from bovine lung. It inhibits several serine proteases such as trypsin, the chemotripsin, plasmin and kallikrein, through the formation of a reversible enzyme-inhibitor complex. By inhibiting kallikrein, aprotinin indirectly inhibits the activation of factor XII, and then the start of coagulation and fibrinolysis induced by blood contact with ‘foreign’ surfaces. Through inhibition of kallikrein, aprotinin also reduces complement activation and renin-angiotensin system and the inflammatory response triggered by kallikrein. Aprotinin does not interfere with platelet function. It is inactive orally and is administered with an initial loading dose followed by continuous intravenous infusion. The enzymatic activity is expressed in units inactivating kallikrein (KIU), 1 mg of aprotinin being equivalent to 7,143 KIU. Concentrations of 125 KIU / mL are necessary to inhibit plasmin and concentrations from 300 to 500 KIU / ml are needed to inhibit kallikrein.
5. EXPERIMENTAL AND CLINICAL STUDY

5.1 MATERIALS AND METHODS

Materials
Tranexamic acid, magnesium stearate, sodium chloride, disodium hydrogen phosphate, and potassium dihydrogen phosphate, sodium carboxymethylcellulose (CMC; 700kDa) were purchased from Sigma-Aldrich (Milan, Italy). Spray-dried lactose was obtained from Polichimica s.r.l (Bologna, Italy). Alginate sodium salt (ALG; low viscosity, 250 centipoise for a 2% dispersion) was purchased from Farmalabor (Canosa di Puglia, BT, Italy). Polyethyleneoxide NF grade (PEO; Polyox WSR-301; 4000 kDa) was purchased from Colorcon (Dartford Kent, UK).

TrA Quantitative Analysis
Tranexamic acid was quantified after derivatization with fluorescamine by UV spectrophotometry.\textsuperscript{32} Briefly, a fluorescamine solution in ethanol (0.5 mg/mL) was added to a TrA solution in phosphate-buffered saline (PBS) (2.38 g Na\textsubscript{2}HPO\textsubscript{4}) × 12 H\textsubscript{2}O; 0.19 g KH\textsubscript{2}PO\textsubscript{4}; 8 g NaCl in 1 L water adjusted at pH 7.4) at a 1:1 vol/vol ratio. After 1-hour incubation in the dark, the sample was analyzed by UV at 392 nm on Shimadzu UV-1204 apparatus (Shimadzu, Milan, Italy) fitted out with a 0.1-cm quartz cell (Hellma Italia, Milan, Italy). Linearity of response was verified in the 5- to 100-µg/mL concentration range (R\textsuperscript{2}=0.999).

Matrix Preparation
Matrices were prepared by direct compression of TrA (50 mg), bioadhesive polymer (ie, CMC, ALG, or PEO) (48 mg), lactose (379.6 mg), and magnesium stearate (2.4 mg) by a manual hydraulic press (Specac Ltd, Slough, UK). All the components were preliminarily milled, sieved through a 90-µm sieve, and mixed in a Turbula apparatus (Willy A. Bachofen AG, Muttenz, Switzerland). Matrices were prepared with a diameter of 1.3 cm (Fig. 2). Their hardness was checked using a handheld tablet.
hardness tester Monsanto type. The instrument measures the force required to break the matrix when the force generated by a coil spring is applied diametrically to the tablet.

**Release Kinetics of TrA**

Release kinetics of TrA from matrices were evaluated according to the dissolution test method, apparatus 1 reported in the European Pharmacopoeia (Ph. Eur), 7th edition. Release was performed in 500 mL PBS at 37°C and 30 revolutions/min. At predetermined intervals, 2 mL was withdrawn and assessed for TrA content as reported above. Results were compared with dissolution profile of TrA powder according to dissolution test method, apparatus 2. The concentration of TrA in the surrounding medium was always below maximum solubility. Results are reported as percentage of TrA released ± SD of 4 replicates.

**Swelling/Erosion Kinetics of Tablets**

Matrix swelling was evaluated in PBS by measuring weight over time. Matrix was secured in a basket made of aluminum net and placed in 10 mL of medium at 37°C. At predetermined intervals, matrices were withdrawn, gently blotted with filter paper, and weighted. Results are expressed as the ratio between matrix weight at time $t$ ($W_t$) and initial matrix weight ($W_0$) ± SD on 4 samples.

Matrix erosion was evaluated under the same conditions as those described above for swelling. A sample was prepared for each testing time. At predetermined intervals, the medium was withdrawn; the matrix was dried up to constant weight at 40°C in a vacuum. The fractional weight loss (WL) of the matrix was calculated using the following equation:

$$WL = \frac{(W_i - W_t)}{W_i}$$

where $W_0$ is the initial weight of the matrix, and $W_t$ is the weight of the matrix at time $t$. The results are expressed as mean ± SD of 4 replicates.
Subjects and Methods

From September 2008 to April 2011, 84 patients undergoing single or dual anticoagulation therapy (48 women and 36 men) from the Department of Neurosciences and Reproductive and Odontostomatologic Science of the University of Naples “Federico II” were selected for the study. The study was a Randomized Clinical Trial.

Patient ages ranged from 39 to 74 years (mean age was 56.5 years). Thirty-four patients (40.4%) had a history of hypertension, 21 patients (25.0%) of ischemic heart disease, and 11 (13.0%) of cerebrovascular disease. In addition, 9 (10.8%) had coronary artery stents, and 9 had (10.8%) cardiac dysrhythmias. Fifty-one patients (60.8%) were receiving single antiplatelet therapy with clopidogrel or ticlopidine. The remaining 33 patients (39.2%) were undergoing dual antiplatelet therapy with aspirin and clopidogrel or ticlopidine.

At the moment of the study, they needed at least 2 dental extractions; they were on oral anticoagulant therapy, and their INR range was from 2 to 4.

The patients were randomized by the flip of a coin into 2 different therapeutic approaches:

- control group (31 patients), where the extractions (157 in total) were performed after suspending the oral anticoagulant therapy a few days before the operation, reaching a patient’s INR less than 2
- study group (53 patients), where the extractions (173 in total) were performed maintaining the patient’s dose regimen of anticoagulant therapy unchanged; the control of hemostasis in the residual socket was carried out by refilling it with a new absorbable swelling sponge loaded with TrA (Figs. 3,4,5,6,7).

The patients’ INR was assessed in the 24 hours preceding oral treatment. In both groups, the extractions were performed with minimal bone trauma and followed by an instrumental screening of the residual bone and a resorbable ‘mattress’ suture.
The postoperative protocol did not include the use of cephalosporins, macrolides, quinolones, which have been shown to have a potential in interfering with the coagulation cascade. To prevent infections, the patients were treated with intramuscular teicoplanin 200 mg once a day and intramuscular Nebcin (tobramycin for injection) 5 mg/kg twice a day at 2 days before and 4 days after surgical procedures. Diclofenac was used as an analgesic for its limited effects on blood coagulation.

We excluded patients having systemic bleeding disorders (von Willebrand disease, hemophilia) or systemic conditions that caused coagulopathies (liver disease). All patients signed an informed consent before participating in the study, which was reviewed and approved by the University institutional review board.

The differences in postoperative bleeding (immediate and delayed) and the need of its treatment at the hospital among the 2 groups were evaluated. $\chi^2$ Test was used to test differences in the prevalence of postoperative bleeding between groups.

5.2 RESULTS

Matrix Loaded With TrA

Three different types of matrices based on spray-dried lactose/mucoadhesive mixtures were prepared by direct compression. CMC, PEO, or ALG were selected as mucoadhesive component. Tranexamic acid-containing matrices displayed a crushing strength around 4 kg, which is a typical value for softer sublingual and chewable tablets.

Matrix swelled without disintegrating up to 60 minutes, thus suggesting that a 10% of the weight of mucoadhesive polymer was suitable to avoid fast matrix disintegration once in contact with an aqueous medium. Furthermore, swelling/erosion profiles highlighted that matrices made of ALG and CMC absorbed a high amount of water in the early hydration stage and increased their weight around 1.5 and 2.5 folds, respectively.
Swelling of PEO was slower and reached its maximum in around 2 hours. After initial water uptake, matrices started eroding because of progressive polymer and TrA dissolution in the aqueous medium. Tranexamic acid is a very hydrophilic drug, and its dissolution is practically instantaneous. On the other hand, the incorporation of TrA in the matrices allowed its slow and sustained release at a rate depending on the type of bioadhesive polymer added. Because of fast swelling and TrA release rate, ALG matrices were considered the most appropriate candidate to test in vivo performance.

**Clinical Study**

All the individuals completed the study, and the protocol achieved good results. There were no significant differences between the groups in the mean age, proportions of men/women, and number of teeth extracted.

Naturally, the mean INR for the anticoagulant group was significantly higher at 2 than that for the control group at 1.6 ($P \leq 0.001$).

No differences in the prevalence of postoperative bleeding were found ($P=0.136$).

There were only 6 hemorrhagic complications (7.2%). Four patients of the control group presented a late postoperative hemorrhage between 2 and 4 days after extraction period-related to the formation of a very large coagulum. The first of these four patients was a 63-year-old woman receiving a dual anticoagulation therapy for a coronary artery stent (INR= 2.8); her hemorrhage was stopped, by making a careful and meticulous curettage of the alveolus, re-suturing under local anesthesia, and applying pressure with gauze moistened with TrA. The same technique was used to control the bleeding of the other 3 patients of the control group who had a history of hypertension.

Two patients of the study group showed an immediate postoperative hemorrhage even after the placement of the matrix that swelled and was retained inside the wound with the suture. A second subsequent correct placement of the new matrix was sufficient to control the bleeding and ensure an
adequate clot formation. In all cases, the study group did not require further hospitalization or systemic therapy.

5.3 DISCUSSION

Recently, several authors suggested that levels of anticoagulation can be maintained and any subsequent post-extraction hemorrhage be treated with local treatments such as suturing and/or in situ application of hemostatic agents. The aim of this strategy is to minimize the risk of adverse thromboembolic events including embolic stroke, myocardial ischemia, or even death. The incidence of thromboembolism due to a short-term interruption of anticoagulation therapy within the 30-day follow-up period was identified as ranging from 0.5% to 1%. To avoid interruption or a reduction of the anticoagulation therapy, thus diminishing the intra-intervention and post-intervention bleeding risk, the same authors demonstrated the clinical advantage of the anti-fibrinolytic effect of TrA solutions. The solution was used as a mouthwash or to soak microfibrillar oxidized regenerated cellulose, where it has proved particularly effective in preventing postoperative bleeding. However, many authors reported how early bone healing after the placement of oxidized cellulose appeared to be impeded. In our research, TrA was integrated in a cellulose swelling matrix able to conform to the tridimensional post-extractive alveolar cavity, ensuring also a mechanical contribution to homeostasis. The design of the hemostatic matrix was carried out taking into account that the matrix should (i) provide intermediate mechanical properties suitable to confer appropriate resistance to the matrix as well as the possibility of cutting it at a size suitable for insertion inside the alveolar cavity, (ii) rapidly swell once in contact with blood forming a muco adhesive plug, and (iii) disintegrate rapidly after TrA delivery. To this purpose, we selected 3 well-known mucoadhesive polymers safely used in pharmaceutical dosage forms. The amount of mucoadhesive polymer was maintained as low as possible to avoid the formation of a slowly swellable glassy hydrophilic matrix releasing the drug at too
slow a rate. On the other hand, the amount of lactose used was maintained high to promote the fast hydration and erosion of the matrix while guaranteeing mucoadhesin and avoiding disintegration. The crushing strength, which is an indicator of the strength required to break a matrix in the diametric compression test, had to be high enough to withstand mechanical stress during packaging, shipment, and handling by the consumer. Values obtained can be considered suitable to attain resistance of the mucoadhesive matrices to abrasion or breakage under conditions of storage, transportation, and handling before usage. Swelling/erosion of the matrices occurred because of matrix hydration, solubilisation of mucoadhesive component, lactose, and drug. Thus, initial water uptake was accompanied by a weight increase and followed by a progressive weight loss of the matrix. Upon hydration, the disentanglement of polymer chains starts to occur as a function of polymer hydrophilicity, with the PEO matrix being the slowest to reach maximum water uptake and erode completely. Furthermore, the addition of a very limited amount of a hydrophilic polymer in the matrix was sufficient to keep it intact over time, for it to absorb a good amount of blood and erode itself by releasing a suitable amount of TrA. The swelling/erosion profiles were directly related to TrA release rate: the faster the erosion of the matrix, that is ALG, the faster the release rate.

Taken together, these results highlight how the compositions proposed, especially TrA-ALG matrix, can perfectly fit an application in the alveolar cavity, where the system should work as a physical plug able to absorb blood, release TrA, and erode very rapidly.

The clinical outcome of the system was very satisfactory, highlighting several advantages of this new delivery system for TrA after extraction in patients receiving anticoagulant therapy. In terms of activity, the TrA-loaded system was able to promote an adequate homeostasis without the suspension or reduction of anticoagulant therapy and, as a consequence, no additional thromboembolic risk.

As a consequence, hospitalization can be reduced with a net benefit in socioeconomic terms. Furthermore, the developed matrices have peculiar swelling (Figs. 8A, B) and mucoadhesive
properties, a reasonable cost and reduced expenses, including hospitalization. Patients had no infectious risk (high compatibility), and owing to its synthetic nature, there is no risk of transmissible disease related to the use of the cellulose sponge as compared with the fibrin adhesive, which derives from a pool of patients.\textsuperscript{36}

The limitations of the present study include the small sample size (n= 84); a larger experimental population may be needed to elucidate the safety of this approach. However, it is clear that the use of an advanced delivery system for TrA with the discontinuation of warfarin therapy and INR in therapeutic range, is safe and effective.

5.4 CONCLUSIONS

Bioadhesive swelling systems loaded with TrA and especially those prepared with ALG due to their peculiar composition, show fast swelling, drug release and erosion, so they are particularly fit for an application as an intra-alveolar medicated plug. Clinical studies demonstrate that the system is therapeutically relevant in patients at risk undergoing systemic anticoagulant therapy.
Fig. 3: Shape and size of the matrix used in the clinical trial

Fig. 4: Extraction with minor bone trauma

Fig. 5: Placing of the loaded matrix in the newly formed oral cavity
Fig. 6: Suturing of the absorbable stitches with the ‘mattress’ technique

Fig. 7: Hemostasis obtained after the operation in the sites under observation

Fig 8A and B: Swelling matrix apply pressure inside alveolar cavity, ensuring an adequate clot formation
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