

The **Astute® Advanced Heparin Coating** has obtained clinical recognition for its successful use on blood contacting medical devices. The non-thrombogenic/anti-thrombogenic **Astute®** system directly negates the adverse biological responses that are initiated when blood contacts a foreign surface, such as platelet/protein adhesion, platelet activation and thrombus formation (blood clot).

## Overview

Advancements in medical device technologies demand that novel, highly efficient materials and coatings be developed in order to enhance the biological acceptance of such materials when in contact with blood.

Indeed, recent developments in devices aimed at the restoration, maintenance and support of diseased organs, vessels and tissues rely heavily on efficient surface properties in order to negate many of the adverse affects that occur when artificial materials are implanted.

At BioInteractions we strive to develop the next generation of biomaterials, advancing healthcare through innovation. Our goal, which we have stood by for the past 18 years, is to contribute positively to improved patient well-being and speedier recovery times through the development of leading edge biocompatible materials and solutions.

In order to develop highly efficient blood-compatible (haemocompatible) coating materials, that will support the function of medical devices used to treat a range of conditions, it is important to understand the fundamental responses elicited by the body when an artificial material/device comes into contact with blood.

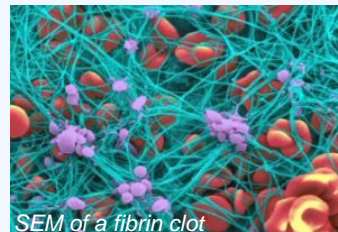
## The Natural Response

The interaction between blood and an artificial surface is a dynamic process, involving proteins, cells and small solute molecules. Once in contact with blood, artificial surfaces rapidly obtain a layer of adsorbed blood proteins, which varies in a complex fashion and is dependent on the surface properties of the artificial material/device in question.

Haemostasis, the combination of processes that ultimately lead to cessation following vascular injury, is the body's natural mechanism for maintaining and restoring integrity throughout the vascular system. However, this process can also elicit undesirable properties in the presence of artificial materials, leading to an aggressive response that ultimately results in the formation of an *in situ* thrombus (blood clot).

The blood clotting process (thrombosis) is the body's natural response to bleeding and involves the principle mechanisms of platelet adhesion, platelet activation and platelet aggregation, as well as fibrin coagulation (polymerisation and stabilisation). This process can also occur when blood contacts an artificial (foreign) material, thus having a detrimental effect on device performance and the patient's health.

The coagulation (clotting) of blood is a complex process that follows a sequential series of events collectively referred to as the blood coagulation cascade. This cascade can follow two pathways that converge and culminate in the formation of a fibrin clot.



Clearly, the development of haemocompatible and biocompatible materials is of paramount importance when implanting artificial devices within the body in order to negate the natural biological responses that are elicited. However, producing bulk materials that display such properties from the outset is extremely difficult and it is more often than not easier and more efficient to apply a coating to the surface of the device to impart these desirable properties.

## Approaches to Haemocompatibility

As a result, academics and industrialists have strived to develop haemocompatible coatings for application on blood-contacting medical devices. This development has led to two schools of thought regarding the best approach to haemocompatibility.

One approach advocates the use of passive "non-thrombogenic" materials and proposes that these surfaces offer the most beneficial interface for blood-material interaction. Non-thrombogenic materials are defined as those that do not enhance protein binding or activate platelets or white blood cells.

The second approach to achieving haemocompatibility is to use active "anti-thrombogenic" materials, such as heparin and heparin-like materials, which take an active role in providing such compatibility.

Heparin has been employed clinically for many years as an anti-coagulant agent used during surgery to prevent blood-clots and in the treatment of various thrombotic disorders. The anti-thrombogenic nature of heparin is based on its ability to bind to and induce a conformational change in the anticoagulant, antithrombin III (ATIII). ATIII is a potent inhibitor of numerous reactions involved in blood coagulation,

demonstrating inhibitory activity towards several serine proteases, including thrombin. Although ATIII displays potent inhibitory activity alone; this activity is greatly enhanced (up to 1000-fold increase) in the presence of heparin.

These two approaches act by affecting different stages in the blood clotting cascade. Non-thrombogenic materials act in a passive fashion at the initial stage, preventing or reducing platelet adhesion. Heparin on the other hand takes a more active role and interacts at various stages of the clotting cascade to prevent thrombus formation.

BioInteractions have developed the Astute® Advanced Heparin Coating, which incorporates both non-thrombogenic and anti-thrombogenic properties on the same polymer backbone. This coating provides triple endothelial-like action that acts in a synergistic fashion to prevent platelet adhesion/activation, whilst also inhibiting the activity of thrombin simultaneously.

### Astute® Advanced Heparin Coating

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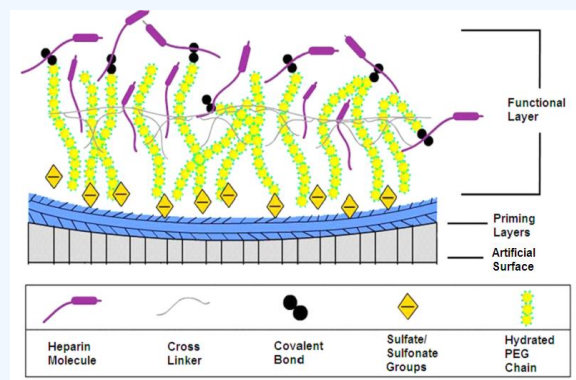
The non-thrombogenic/anti-thrombogenic Astute® system directly negates the adverse biological responses that are initiated when blood contacts a foreign surface, such as platelet/protein adhesion, platelet activation and thrombus formation (blood clot).

The novel non-thrombogenic/anti-thrombogenic polymer coating provides the triple endothelial-like action. Astute® does this through three components: heparin, negative charge and hydrophilicity.

The non-leaching heparin molecule is covalently linked to the surface and displays extremely high heparin activity to provide similar beneficial effects to heparan sulfate, present in the natural endothelium.

Sulfate and sulfonate groups, which carry a strong negative charge (heparan sulfate is similarly a heavily sulfated molecule with a strong negative charge), are incorporated into the functional layer of Astute® to repel red blood cells and proteins.

Highly hydrophilic poly(ethylene glycol) (PEG) chains are covalently linked within the biosurface and help to minimise the interaction between the device surface and blood elements, by promoting laminar flow and reducing turbulence, thus reducing protein/cellular deposition.



Schematic representation of Astute®.

### Biochemical Properties and Performance

The non-thrombogenic component of Astute® is specifically designed to reduce the initial protein/platelet adhesion that occurs when an artificial material comes into contact with blood. The highly hydrophilic PEG chains and negatively charged sulfate and sulfonate groups effectively lessen non-specific protein binding and thus, markedly reduce platelet adhesion.

### In-Vitro Evaluation

This property has been evaluated *in-vitro* using flow cytometry and radioactive Indium ( $In^{111}$ ) to radiolabel platelets. The adhesion of the radiolabeled platelets to an Astute® coated sample, relative to an uncoated sample, was determined and revealed a significant reduction in platelet adhesion of 95% for the Astute® coated sample.

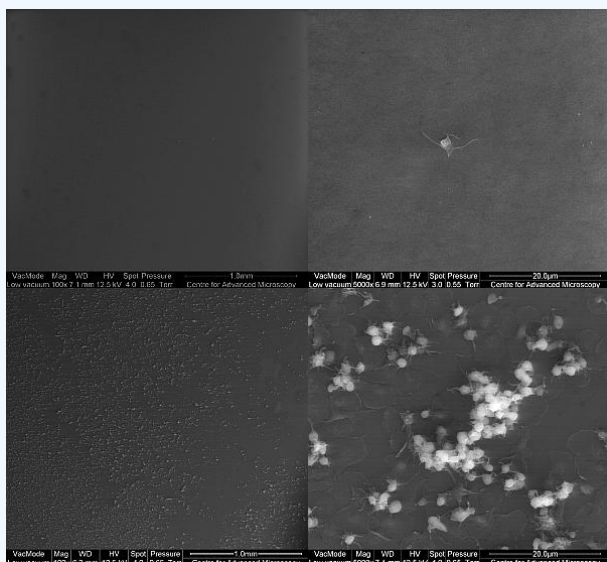
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Scanning Electron Microscopy of coated (top) and uncoated (bottom) surfaces after Platelet Adhesion Studies

When platelets become activated, they change their shape and release several physiologically active substances which can lead to platelet aggregation, a platelet plug and ultimately, thrombus formation. Therefore, determination of how the Astute® coating effects platelet activation is an important test.

An *in-vitro* blood loop has been employed to study how the Astute® coating effects platelet activation. In this test a coated and non-coated poly(urethane) catheter sample was compared and the platelet activation markers PF-4 and  $\beta$ -thromboglobulin were assessed via an ELISA based assay. A significant reduction of 92% was observed for the Astute® coated samples.

An *in-vitro* blood loop study has also been used to determine inflammation and complement activation. Again, coated and non-coated poly(urethane) catheter samples were evaluated and an ELISA based assay was employed. In order to determine how the Astute® coating affects inflammation and complement activation, the markers P-Selectin and SC5b-9 were studied. Overall, a 90% reduction in these markers was observed for the coated samples in comparison to the non-coated ones.

The *in-vitro* blood loop study also revealed a lack of occlusion within the coated device as evidenced by a well maintained flow rate throughout the duration of the experiment.

The biologically active component of Astute®, heparin, has been specifically incorporated into the coating to take an active role in thrombo-resistance, much like the natural endothelium. Heparin can be evaluated *in-vitro* via several methods to determine just how 'active' this molecule is. Chromogenic assays, which measure FXa inhibition, have long been employed to determine the activity of immobilised heparin. The Astute® coating currently displays the highest heparin activity on the market when compared to alternative products, with a value of 250-300 mU/cm<sup>2</sup>.



Internal surfaces of coated (top) and uncoated (bottom) samples used during blood loop study.

This heparin activity can be backed up by measuring the antithrombin uptake using a modified FXa assay. Here, the antithrombin uptake has been measured at  $93 \pm 2.8$  pmoles/cm<sup>2</sup> ( $I = 0.15$ ) and  $33 \pm 1.3$  pmoles/cm<sup>2</sup> ( $I = 0.4$ ).

These *in-vitro* evaluations highlight the significant advantage of using Astute® for reducing both platelet adhesion and platelet activation, as well as inflammation and complement activation and may therefore prove beneficial when used *in-vivo*.

### In-Vivo Evaluation

In order to evaluate the efficacy of the Astute® coating *in-vivo* a number of evaluations were performed, including thrombus formation and device occlusion (for tubular device, e.g. catheter).

An ovine model was selected and 6 sheep were used. Random placing of coated and non-coated poly(urethane)

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catheters in the left and right internal jugular veins was carried out and implantation was for a 30 day period during which simulated dialysis sessions were performed.



*Uncoated and coated samples explanted after 30 days.*

Gravimetric analysis after the animal study revealed a 98.4% reduction in thrombus mass for the Astute® coated samples. Again, a lack of occlusion was observed within the coated device during and after the animal studies.

This result highlights an important property of the Astute® coating process, that the internal surfaces of devices, e.g. catheters, can be coated as effectively as the external surfaces, thus providing complete haemocompatibility over the entire device.

### Coating Stability

The stability of Astute® has been demonstrated by a series of mechanical, thermal and chemical tests.

The 'wet glove' test is used to simulate mechanical abrasion, which is indicative of the point of use, i.e. a catheter being tunnelled through the skin. Astute® remains intact and unaffected (as assessed by Toluidine blue dye and heparin assay).



*Poly(urethane) catheter coated with Astute® and subjected to the 'wet glove' test. Toluidine blue dye indicates a homogenous coating that has remained intact.*

Ageing studies to determine the shelf-life of the coating were performed. After 6 months actual ageing and 1 year accelerated ageing, both in humid and non-humid conditions, no significant drop in heparin activity was observed.

Astute® is also unaffected by ethylene oxide (EtO) sterilisation.

The Astute® coating has demonstrated excellent long-term chemical stability *in-vivo*.

### Summary

The Astute® Advanced Heparin Coating provides superior haemocompatibility by preventing protein and platelet deposition, platelet activation and greatly reducing thrombus formation. This is achieved thanks to the non-thrombogenic/anti-thrombogenic nature of Astute®, which provides triple endothelial-like action on the same polymer backbone – it is not just a heparin coating!

The Astute® coating suits an extremely broad range of medical devices and substrates in a cost-effective manner.

Clinical applications include cardiovascular, perfusion, infusion, neuro-stimulation, cardio-stimulation and organ support.

The coating process is simple, water-based and uses no harsh solvents. Coating is carried out at room temperature, in less than 30 minutes. A flexible dip-coat batch process is employed, which can be easily tailored depending upon the device being coated. The coating is hydrophilic and promotes laminar flow.

***BioInteractions has established license agreements with major medical device manufacturers for the Astute® coating technology. These include Medtronic Cardiac Surgery (Trillium® Biopassive Surface) and Covidien (The Tal Palindrome™ Emerald™ and Sapphire™ Hemodialysis Catheter Range)***

***We are committed to the advancement of healthcare through innovation and welcome interest in the Astute® Advanced Heparin Coating.***

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