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Indwelling catheters and medical implants with FXIIIa inhibitors: a novel approach to the treatment of catheter and medical device-related infections

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Abstract

Central venous catheters (CVCs) are being utilized with increasing frequency in intensive care and general medical wards. In spite of the extensive experience gained in their application, CVCs are related to the long-term risks of catheter sheath formation, infection and thrombosis (of the catheter or vessel itself) during catheterisation. Such CVC-related-complications are associated with increased morbidity, mortality, duration of hospitalisation and medical care cost [1].

The present study incorporates a novel group of Factor XIIIa (FXIIIa, plasma transglutaminase) inhibitors into a lubricious silicone elastomer in order to generate an optimized drug delivery system whereby a secondary sustained drug release profile occurs following an initial burst release for catheters and other medical devices. We propose that the incorporation of FXIIIa inhibitors into catheters, stents and other medical implant devices would reduce the incidence of catheter sheath formation, thrombotic occlusion and associated staphylococcal infection. This technique could be used as a local delivery system for extended release with an immediate onset of action for other poorly aqueous soluble compounds.

Keywords

Central venous catheter; catheter-related complications; catheter-related infection; catheter-related thrombosis; transglutaminase; FXIIIa inhibitor; silicone catheter

Introduction

Between 2.8 – 18 % of CVC patients suffer from catheter-related infection [2]. In the United States, catheter-related bloodstream infections (CRBSI) occur with 3 - 7 % of catheters and affects more than 200,000 patients per year with a mortality of 10 - 25 % and an extra cost to the health-care system of approximately \$25,000 per episode [3]. In the United Kingdom, almost 6000 patients acquire catheter-related blood stream infections every year, resulting in an increase in the length of hospitalisation and medical care costs [4], a number that is increasing rather than decreasing. Furthermore, the mural thrombi may partly or entirely block the blood vessel and may involve 12 - 74 % of all CVCs [5]. The wide variability observed is due to the different diagnostic methods used, difficulties in interpretation of observations, the way thrombosis is defined and reported [6], variation in catheter type, position, duration of insertion, and the underlying diseases [5]. Aside from reducing the

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function of the catheter, the CVC-related thrombi can cause postphlebitic syndrome in 15 - 30 % of cases and pulmonary embolism in 11 % of cases (only half of which are symptomatic) [5].

Many approaches have been used to reduce the incidence of catheter-related complications including: regular flushing with heparin and saline [5], administration of warfarin or low molecular weight heparin (LMWH), use of antiseptic-coated catheters, antibiotic-coated catheters or silver-impregnated catheters [7]. The efficacy of low-dose warfarin prophylaxis remains debatable, although many earlier studies support the use of low-dose warfarin, most recent studies do not. There is also a major inconvenience of daily subcutaneous injections of standard heparin or LMWH. Even low prophylactic doses of LMWH may accumulate and lead to bleeding in the asthenic or elderly cancer patient with a reduced glomerular filtration rate, an effect that is augmented in patients with reduced renal function. Nevertheless, in patients with sufficient nutrition and hepatic function, the risks of this approach seem negligible [5]. There have been debates concerning the utilization of antibiotic-coated catheters and the increased risk for bacterial resistance and the possible ineffectiveness of these agents against antibiotic resistant nosocomial bacteria and fungi. To date, clinical trials have not overcome this problem [8,9].

Transglutaminases are enzymes that cross-link proteins through an acyl-transfer reaction between the γ -carboxamide group of peptide-bound glutamine and the ϵ -amino group of peptide-bound lysine, resulting in a ϵ -(γ -glutamyl) lysine isopeptide bond [10]. Blood coagulation Factor XIII (FXIII), also known as fibrin-stabilizing factor (or fibrinolygase), is one of the best characterized transglutaminases, and its physiological role is well established [11]. FXIII is a tetramer composed of two A subunits (FXIII A) and two B subunits (FXIII B) noncovalently associated [12]. Dissimilar to many other TGs, it is a zymogen (proenzyme) that is converted into an active transglutaminase (FXIIIa) by the proteolytic action of thrombin and Ca^{2+} in the final phase of the coagulation cascade. Its main task is to crosslink α - and γ -chains of fibrin and α_2 -plasmin inhibitor to fibrin. By this way FXIIIa strengthens fibrin and protects it from the prompt elimination by the fibrinolytic system [13]. Cross-linking by FXIIIa improves the mechanical strength, rigidity and elasticity of the clot and increases its resistance to plasmin-mediated degradation [11]. Therefore, inhibition of FXIIIa activity enhances fibrin degradation mediated by plasmin *in vitro* and accelerates thrombolysis in animal models of venous and arterial thrombosis and in experimental pulmonary embolism [14].

FXIIIa is also exploited by Staphylococci which become permanently attached to the blood clot, thus shielding them from immune attack and the antibiotics used to eradicate them. The link between catheter-related thrombus formation and Staphylococci infection is the mechanism by which *Staphylococcus aureus* colonise surfaces of medical devices by binding to the host proteins fibrin/ fibrinogen and fibronectin. The interaction is mediated by the production of a number of microbial surface components recognizing adhesive matrix molecules; in *Staphylococcus aureus* these include the fibrinogen-binding clumping factors A and B and the fibronectin-binding protein (FnB) [15]. FnB is a substrate for FXIIIa and undergoes covalent cross linking to fibrinogen and [16,17]. *Staphylococci aureus* becomes covalently cross-linked to fibrinogen and fibrin during deposition within the fibrin-platelet matrix of thrombi on the catheter surface; this prevents the release of bacteria into the blood during natural thrombolysis and retaining the organisms in an environment protected from antibiotics action and host defenses [18].

We have recently introduced a novel group of transglutaminase inhibitors [19,20,21]. These small, non-toxic inhibitors could prevent stabilisation of thrombi by FXIIIa and consequently increase the natural rate of thrombolysis. In addition they could reduce

staphylococcal colonisation of catheters by inhibiting FXIIIa-mediated cross-linking of staphylococci to host proteins on the catheter surface (Griffin et al., 2004; Lambert, 2007) [18, 19]. The major aim of this study was the integration of the fluorescent FXIIIa inhibitor AM2/97 (Fig. 1A) into silicone central venous catheters, with the intent of producing CVCs with a lower incidence of thrombosis and related staphylococcal infections.

Materials and methods

Materials

MED5-6382 medical grade silicone elastomer (three component silicone: Base, cross-linker and catalyst) was obtained from Nusil Technology (Carpinteria, USA). Sodium bicarbonate and phosphate buffered saline (PBS) were acquired from Sigma-Aldrich (Dorset, England). Citric acid was purchased from VWR international Ltd. Glass spacer plates were procured from Bio-Rad Laboratories, Inc. Unless stated otherwise PBS was used at 0.01 M, pH 7.4. Doubly distilled and filtered water was used in the preparation of all solutions. The silicone elastomers used in this study were manufactured by linear, hydroxy-terminated poly(dimethylsiloxane) macromolecules crosslinked with a low molecular weight tetra (alkyloxysilane) crosslinking agent (TPOS), derived from propanol, in the presence of stannous octoate as a catalyst, via a condensation cure mechanism. AM2/97 and non-fluorescent FXIIIa inhibitors R281 and R283, were prepared within the chemistry department of Aston University as previously described [20].

Effect of FXIIIa inhibitors on release of *Staphylococcus aureus*

The role of these novel FXIIIa inhibitors in enhancing the natural rates of thrombolysis and reducing the colonization of catheters by staphylococci was assessed *in vitro* as follows: Fresh human venous blood (1 ml) was collected by venepuncture into sodium citrate (13 mM final concentration). After addition of *Staphylococcus aureus* NCTC 8325 (to 10^6 cfu/ml), tissue plasminogen activator (TPA, to 100 ng/ml), aqueous solutions of R281 and R283 (500 μ M) or water (control), or AM2/97 (fluorescent-labeled FXIIIa inhibitor,) dissolved in 0.1% DMSO with 0.1% DMSO as control was added and the blood was then clotted by addition of CaCl_2 (to 20 mM) and allowed to cross-link for 60 min at 37 °C. Blood clots were washed three times each in 1ml sterile phosphate buffered saline (PBS), resuspended in 1 ml PBS containing 10 mg/ml TPA and incubated with shaking at 37 °C. Samples of the clot suspending fluid were withdrawn at intervals and measured for red blood cell content (absorbance 750 nm) and release of *S. aureus* (by viable counting).

Biological activity of FXIIIa inhibitor

FXIIIa activity was measured using an enzyme linked sorbent assay (ELSA) to measure the covalent incorporation of biotinyl-5-pentylamine into N,N'-dimethylcasein as described previously [22]. Briefly, a 96-well microtitre plate was coated overnight with N,N'-dimethylcasein (10mg ml⁻¹ in 50mM Tris HCl, pH7.5) and a reaction mix containing FXIIIa (2 ng ml⁻¹), biotinyl-5-pentylamine (132 μ M), CaCl_2 (5 mM) and DTT (5 mM) with varying concentrations of inhibitor, was added to each well and incubated for 1 hour at 37 °C. The amount of incorporated biotinyl-5-pentylamine was quantitated by reaction with Extravidin-peroxidase and colour developed with o-phenylenediamine. The IC₅₀ was expressed as the inhibitor concentration at which 50 % inhibition of FXIIIa activity was observed [20].

Preparation of silicone elastomer strips: sustained drug release

Medical grade condensation cure silicone elastomers are conventionally manufactured by the cross linking of α,ω -hydroxyl functionalised poly(dimethylsiloxane) with

tetrapropoxysilane (TPOS) (Fig. 1B). The conventional silicone elastomer mix was prepared by thoroughly mixing 2.5 parts by weight of TPOS as cross-linker, 97 parts of MED5-6382 silicone elastomer as base and 0.5 parts of stannous octoate as catalyst for two minutes. Different percentages of citric acid (CA) and sodium bicarbonate (SB) (0 % (blank), 5 %, 15 %, 30 % and 40 % w/w) in a ratio of 1:3 as additives, together with the FXIIIa inhibitor (AM2/97) (0.5 % and 1 % w/w) were incorporated into the mixture. The formulations prepared are listed in Table 1.

The silicone elastomer mixture was then placed between one glass spacer plate with 1.0 mm integrated spacers, with a size of 10.1×7.3 cm, and a short plate, with a size of 10.1×7.3 cm. The glass plates were left overnight at room temperature to de-aerate. The glass plates used were initially treated with an anti-static foam cleanser and a supergliss spray as a lubricant to avoid adherence of silicone mixture. The elastomeric silicone sheets thus produced were cut into strips of dimensions 10×30 mm using a scalpel and subsequently weighed (mean weight = 0.42 ± 0.08 g, mean surface area = 3.00 cm^2).

Production of a smart polymeric coating on silicone strips: initial immediate drug release

In order to obtain an immediate onset of action of drug, the silicone elastomer strips were then dip-coated with poly-vinyl-pyrrolidone (PVP). In brief, the polymer was dissolved in chloroform at a concentration of 5 % (w/w). Afterwards, 0.25 % (w/w) of the enzyme inhibitor was added to the polymer solution. The coating solution was kept on dry ice to prevent evaporation of the organic solvent and a subsequent increase in the polymer concentration. Subsequently, the silicone strips were vertically dip-coated in the polymer solution containing the inhibitor and withdrawn three times. The strips were coated using a two, three and four times dip-coating procedure to achieve a dense and regular polymer coating. The coated silicone strips were dried at room temperature to allow the solvent to evaporate completely. The reproducibility of the lifting velocity and coating times was confirmed to yield approximately the same coating thickness.

FXIIIa inhibitor release from the silicone elastomer strips *in vitro*

Each of the silicone strips was placed in a 50 ml universal tube containing 10 ml of 0.01 M phosphate buffered saline (PBS, pH 7.4). The tubes were then put in a shaking water bath set at 80 rpm and 37 °C. The release medium was sampled and replaced every 24 hours (to promote sink condition) over a period of 30 or 40 days. Fluorescence within the release media was quantified using 200 μl aliquots taken every 24 hours and measured spectrofluorimetrically with an excitation maximum at 330 nm and emission peak at 570 nm (Spectra Max Gemini XPS, Molecular Probes). Accordingly the concentration of the inhibitor released within the media was calculated using a drug calibration curve which was previously prepared from a set of standard samples of known concentration. The results were then reported as cumulative drug release, which corresponds to accumulation of the drug within the release media from time zero up to the measured time point, expressed as percentage (%) of total drug added and concentration ($\mu\text{mole/ml}$) of the inhibitor released. Estimation of the amount of the inhibitor entrapped within the coatings through the dip-coating method was calculated by measuring the density of the coating solution and weight changes of the solution subsequent to each coating, which was followed by calculating the number of moles of the inhibitor incorporated per coating.

Biological activity of released FXIIIa

The biological activity of the released inhibitor from the silicone elastomer strips in inhibition of human FXIIIa was verified using ELSA as previously described, using aliquots of 200 μl which were collected from the release medium at preset incubation time points (one day and one, two, three and four weeks). The results were then expressed as a

percentage of remaining FXIIIa activity compared to the negative control (without the inhibitor).

Morphological analysis

Scanning electron microscopy (SEM)—The specimens were thoroughly dried and gold coated by a gold sputter coater (Emscope SC500 Sputter coater, Ashford, Kent, Great Britain) prior to SEM. Cross-sectional SEM images from silicone elastomer formulations were captured by a scanning electron microscope (Cambridge Instruments, Stereoscan 90) at 29 ×, 49 × and 103 × magnification. The experimental conditions involved 0 %, 5 %, 15 % and 30 % (w/w) CA/ SB incorporated formulations, and before and subsequent to incubation (1 day, 1 week and 1 month time points) in PBS.

Optical microscopy—Optical images were obtained from the surface of the silicone elastomer specimen, using a Reichert-Jung Polyvar MET optical microscope with a JVC KY-F75 digital camera, at 25 × and 50 × magnification.

Mechanical properties

Tensile strength—To investigate the greatest lengthwise stress the silicone strips could withstand without breaking, the tensile properties of the strips were measured using a Hounsfield Universal Tester S Series H10KS-0393 (Tinius Olsen Ltd, Surrey, UK) with Q-Mat 2.18 software including Test Generator, Testzone and File examination. The crosshead was able to move at jog speeds of 0.001 to 1000 mm/min, with an extension accuracy of ± 0.01 mm and a speed accuracy of $\pm 0.05\%$ of the set speed. Initially, the test specimens were cut into equal strips, each measuring $30 \times 10 \times 1$ mm. A silicone strip was then held tightly between two clamps while the crosshead moved upwards at a rate of 50 mm/min. The specimen tensile strength was calculated by dividing the amount of force exerted on the specimen at break (Newton) by the cross sectional area of the specimen (mm^2) and expressed in MPa.

Young's modulus—Strain at break was calculated by dividing the extension of the sample at break by its original length. As the force-time profile for all samples was essentially linear throughout, therefore the Young's modulus was calculated by dividing the tensile strength at break by the strain at break.

Statistical analysis

For all experiments, means and standard deviations were calculated and represent one of at least three separate experiments undertaken in triplicate, unless stated otherwise. To determine statistical significance in resultant data, a one-way analysis of variance (ANOVA) was performed. Differences described as significant or extremely significant in the text correspond to $p < 0.05$ or $p < 0.001$ respectively. Tukey's post hoc test was conducted to determine which conditions differ significantly from each other.

Results and discussions

Biological activity of FXIIIa inhibitor

The efficacy of AM2/97 in the inhibition of FXIIIa was assessed by ELSA. Fig. 1C shows that AM2/97 inhibits FXIIIa in a concentration dependent manner. The half maximal inhibitory concentration (IC_{50}) of AM2/97 for FXIIIa was measured at $2 \mu\text{M}$, which is comparable to the non dansylated 283 ($5 \mu\text{M}$) and 281 ($8 \mu\text{M}$) [20].

Effect of FXIIIa inhibitors on release of *Staphylococcus aureus* from human thrombi

There was an increased release rate of *S. aureus* NCTC 8325 from human thrombi formed in the presence of either R281, R283 or AM2/97 (Fig. 2A and 2B). This effect is likely due to the inhibition of *S. aureus* cross-linking to the thrombus proteins [16,17], and increased physical breakdown of the thrombus by inhibition of fibrin-fibrin and/or fibrin-alpha(2)-plasmin inhibitor cross-linking [14].

Influence of additives on release behavior

The release of insoluble or low soluble drugs from silicone carriers can be enhanced by the use of additives. Accordingly, sodium bicarbonate (SB) and citric acid (CA) were added to the silicone formulations. Water-soluble CA and SB forms channels and pores in and on the specimen and react upon mixing to generate carbon dioxide in the channels. The gaseous carbon dioxide as a driving force accelerates the release of the inhibitor [23]. AM2/97, a poorly soluble drug, was not released from the silicone carrier when it was dispersed in the silicone devoid of additives (0 % CA and SB). However, release of the enzyme inhibitor increased correspondingly with incorporation of CA and SB in the silicone strips (Fig. 3A). The amount of released inhibitor from the formulations containing 30 % (w/w) CA and SB powders was greater than the amount released from the preparations containing 15 % and 5 % (w/w) CA and SB powders, respectively (Fig. 3A). Similarly, the amount of release from formulations containing 15 % (w/w) CA and SB powders was more than that of 5 % (w/w) formulation ($P < 0.05$, ANOVA).

Silicone elastomers could not be formed with the formulation incorporating 40 % (w/w), as a result of the high powder content in the specimen.

Additionally, increasing the amount of AM2/97 loaded in the preparation (from 0.5 % to 1 % w/w) at 30 % (w/w) CA and SB significantly improved the duration of release from 30 days to 40 days and also total amount of release, but caused a decline in the percentage of drug release from 26.27 % to 21.75 %, after 40 days (Fig. 3B). This could be due to the low solubility of the inhibitor.

In all preparations, the rate of drug release decreased regularly for the first 14 days. Afterwards, a slow and constant drug release could be observed for all formulations incorporating CA and SB powders. If the drug concentration gradient remains constant, for instance where solid drug particles are present and constant dissolution maintains the concentrations of the drug in solution, the rate of drug release does not vary with time and zero-order controlled release is attained. However, in the present study, regardless of the drug's physical state in the polymeric matrix, this device does not provide zero-order drug release properties. This is because as the drug molecules at the surface of the device are released, those in the centre of the device have to migrate a greater distance to be released. This increased diffusion time results in a decrease in the release rate from the device with time [24].

Initial immediate drug release

In some cases, an initial burst release of drug followed by a secondary sustained drug release is favored. To achieve this after manufacturing, the silicone elastomer strips were dip-coated with PVP by a solvent casting technique.

The incorporated inhibitor showed an immediate drug release within the first 5 minutes of much of the loaded drug (Fig. 3C). For those systems coated twice with the PVP coating, 100 % release was achieved within 30 minutes, whilst maximum amounts of released drug were around 80 % and 70 %, for 3 × and 4 × PVP coating, respectively. Initially, the drug

release may be controlled by the dissolution rate of polymer coating over time. Upon contact of the polymer coating of the silicone strips with PBS, a rapid release of the incorporated inhibitor from the surface on hydration and consequently dissolution of the biodegradable polymer was achieved. Furthermore, it can be concluded that increasing the number of coatings does not affect the release time but affects the concentration of drug released into the media, due to the higher total amount within the coat.

Biological activity of the released inhibitors

The biological activity of the released AM2/97 from the silicone elastomer strips in inhibition of human FXIIIa for a period of one month was confirmed by ELSA (Table 2). It can be concluded that AM2/97 is still biologically active after the release from the silicone elastomer strips, confirming that casting and storage had no adverse effects.

Scanning electron microscopy

To investigate the morphology of the silicone strips prior to and after immersion in PBS, SEM analysis of the strips was performed.

Cross sectional SEM images (Fig. 4) captured from the silicone elastomer strips containing 5 %, 10 % and 30 % CA/ SB prior to and after one month incubation in PBS (37 °C, pH 7.4) confirmed the formation of channels and pores inside the formulations. As the percentage of incorporated CA/ SB was raised, the number and size of pores and channels generated as a result of generating a larger amount of carbon dioxide was increased. The amount of channels and pores at 30 % CA/ SB integrated preparations was considerably higher than that of formulations containing 0 %, 5 % and 15 % CA/ SB. In contrast, in 0 % incorporated CA/ SB (blank), no channels or pores were observed. As previously stated, SB and CA create channels and pores upon mixing and the high pressure caused by the consequent production of carbon dioxide eventually leads to sustained release of the inhibitor.

Similarly, the production of pores and channels in specimens was found to be directly proportional to the length of incubation time. As a consequence, there is a greater amount of pores and channels in the one-month incubated strips as opposed to the one-day and one-week incubated specimens, for all the formulations tested. This could be attributed to the creation of more and larger channels and pores over time.

Macroscopic morphology

Macroscopic photographs of the silicone elastomer strips containing 0 %, 5 %, 10 % and 30 % CA/ SB were obtained prior to and after one month incubation in PBS (37 °C, pH 7.4). Across all the formulations tested, there was no major difference in the appearance of the strips before the incubation (Fig. 5A). However, after one-month immersion in PBS, the CA/ SB incorporated strips (Fig. 5B) gently swelled, while retaining the initial shape. As the amount of amalgamated CA/ SB in the silicone matrix was increased from 5 % to 30 %, the strips were more swollen and expanded. The significant expansion of strips incorporating 30 % CA/ SB compared to the other formulations and blank (0 % CA/ SB) was also visible to the naked eye. These results confirm the absorption of water by the strips integrating CA/ SB as a consequence of the creation of channels and pores during CO₂ production. The appearance of the silicone elastomer strips containing 0 % CA/ SB after one month immersion in PBS portrayed no noticeable alteration or swelling, indicating that channels and pores were not created under these conditions.

Mechanical properties of silicone strips

Tensile strength—The tensile strength of silicone strips prepared in these studies was measured after initial preparation in PBS at 37 °C (Fig. 6) to investigate the greatest lengthwise stress that the silicone strips could withstand without breaking.

Catheters can be exposed to significant tensile force upon removal from the patient which might result in destruction or detachment of the shaft from the balloon in the case of Foley catheters or in the separation of the catheter into two or more parts. Consequently, the elastic recovery of the constituent biomaterial is a necessary property that must be considered when manufacturing catheters [25,26,27]. Commonly, silicone has a tensile strength of 2.4 - 7 MPa and a percent elongation of 350-600 % [27]. According to our results (Fig. 6A), there was no a considerable variation (ANOVA, $P > 0.05$) in terms of tensile strength amongst the blank formulations (0 % CA/ SB) within the range of time points measured (approximately 5 MPa). For the rest of the formulations tested, a common trend apparent is that with an increase in percentage of incorporated CA/ SB, the maximum force required to break the strips and hence the tensile strength was reduced. Across all the time points, the specimens incorporating 30 % CA/ SB demonstrated the lowest tensile strength, whereas the ones with 0 % represented the highest. From these findings it could be suggested that incorporation of more CA and SB powders in the formulations results in the weakening of intermolecular, secondary forces (Van der Waals forces) in the polymer structure.

Moreover, all the silicone elastomer strips illustrated a greater tensile strength prior to incubation than after incubation in PBS. As the incubation period for the formulations amalgamated with CA and SB increased from one day to one month, the tensile strength decreased. For the preparations incorporating 5 % and 15 % CA/ SB, there was a downward trend in the tensile strength from 4.75 to 2.45 MPa and from 3.88 to 1.76 Mpa, respectively, over the entire incubation period. The greatest reduction in the amount of tensile strength at 30 % CA/ SB was on the first day (from 3.33 to 1.32 MPa), after which it remained constant. This could be another consequence of the formation of holes and pores, as a result of gas generation upon the mixing of carbonate and acid powders. Hence, the longer the period of time that the specimens were maintained in PBS, the greater number of pores and channels were formed.

Young's modulus—To quantify the elasticity or stiffness of the silicone elastomers, the Young's modulus (E) of each silicone strip was measured (Fig. 6B). The Young's modulus and consequently the elasticity of the silicone elastomer strips declined steadily, with an increase in the amount of incorporated CA/ SB in the formulations from 0 % to 30 %. The highest modulus, ~ 0.110 MPa, was measured in the preparations incorporating 0 % (w/w) CA/ SB which were the most elastic of all the specimens. Conversely, the smallest modulus, 0.06 -0.08 MPa, was measured in 30 % CA/ SB (w/w) integrated formulations, indicating the lowest elasticity and highest stiffness among the formulations.

There was no considerable difference (ANOVA, $P > 0.05$) in the Young's modulus measured in all the preparations devoid of CA/ SB (0 %), prior to and after incubation in PBS (~ 0.110 MPa). For the rest of the formulations (5 %, 15 % and 30 % CA/ SB), the amount of applied force and stress which was required to break the strips, in addition to Young's modulus, declined slowly with increased incubation (day to month), particularly at 30 % CA/ SB (w/w), where the Young's modulus declined gradually from 0.085 MPa to 0.063 MPa. However, the change in the Young's modulus was insignificant (ANOVA, $P > 0.05$) when comparing day zero release with that of one day release and also when comparing the time point after one week release with that after one month for 5 % and 15 % CA/ SB incorporated formulations. The creation of channels and pores in the CA/ SB amalgamated preparations during the suspension of strips in PBS weakens the

intermolecular forces that act between molecules or between functional groups of macromolecules, in turn lessening the elastic properties of the strips and enhancing stiffness.

In a similar study conducted by [28], the tensile strength of the silicone strips measured from 1.46 – 3.37 MPa and the Young's modulus measured from 0.68 – 2.26 MPa. The resultant tensile strength was in accordance with the experimental results obtained from Fig. 6A, while the elastic modulus was greater than the comparative study conducted in this chapter (Fig. 6B). This could be attributed to the incorporation of CA/ SB in the formulations as earlier discussed.

Conclusion

The results presented demonstrate that the FXIIIa inhibitors used in these studies are capable of increasing the rate of release of *Staphylococcus aureus* from the fibrin clots thus preventing build up of the bacteria in the fouled catheters and making them available to the host immune system and drug treatment. Studies on the release of the inhibitor from the silicone carrier indicated it was not released from the carrier when it was dispersed devoid of additives. However its release profile was improved by using CA and SB as additives to form channels and pores upon dissolution and generate gaseous carbon dioxide as a driving force to accelerate the release of the inhibitor. The drug release rate could be controlled by the amount of CA and SB incorporated in the silicone matrix. A common trend apparent was that with an increase in the percentage of incorporated CA and SB, the consequent drug release also increased. Thus, the amount of the inhibitor released at 30 % CA and SB is the most amongst the formulations and approximately 30 fold higher when compared to the control. Morphological analysis confirmed the formation of channels and pores inside the specimens upon the addition of CA and SB. Furthermore, it was found that the inhibitor was still biologically active subsequent to being released from the silicone elastomer strips for up to 1 month. However, the tensile strength, in addition to Young's modulus of silicone elastomer strips, decreased constantly with an increasing amount of amalgamated CA/ SB in the formulations. According to these preliminary studies, FXIIIa inhibitors when incorporated into catheters and/or other medical devices could offer new perspective for preventing biomaterial-associated fibrin fouling and *Staphylococcus aureus* infections.

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Figure 1.

A: FXIIIa inhibitor (AM2/97). **B:** Cross-linking chemistry between hydroxy-terminated poly(dimethylsiloxane) and tetrapropoxysilane (TPOS) in the production of condensation cured silicone elastomer (drawn using Chemschetch software). **C:** The Inhibition of FXIIIa by AM2/97. Activity was determined using an enzyme linked sorbent assay (ELSA). The results represent mean $\pm$ SD, n = 8.

Figure 2. Effect of FXIIIa inhibitors on release of *Staphylococcus aureus* NCTC 8325 from human thrombi. **A:** Inhibitors R281 and R283. **B:** fluorescent inhibitor AM2/97.

Figure 3.

A: Cumulative release as a percentage of total added fluorescent FXIIIa inhibitor (AM2/97) into 10 ml PBS from silicone elastomer strips over 30 days under sink condition. The formulations incorporated 0 %, 5 %, 15 %, 30 % (w/w) CA: SB, and 0.5 % (w/w) AM2/97. **B:** Cumulative release as a percentage of total added fluorescent FXIIIa inhibitor (AM2/97) into 10 ml PBS from silicone elastomer strips over 40 days under sink condition. The formulations were incorporating 30 % (w/w) CA: SB, 0.5 % and 1 % (w/w) AM2/97. **C:** Cumulative release as a percentage of total added fluorescent FXIIIa inhibitor (AM2/97) into 10 ml PBS from silicone elastomer strips with 2, 3 and 4 × PVP coatings over 60

minute under sink condition. The formulations contained 5 % (w/w) PVP and 2.5 % (w/w) AM2/97. All results denote mean $\pm$ SD from 3 independently synthesized batches.

Figure 4.

Cross sectional SEM images from silicone elastomer strips containing 0 %, 5 %, 15 % and 30 % CA: SB respectively from left to right, prior to (A) and after (B) 1 month incubation in PBS (37 °C) at 49 × magnification.

Figure 5. The appearance of silicone elastomer strips incorporating 0 %, 5 %, 15 % and 30% CA/ SB before (A) and after (B) one-month incubation in PBS. All bars = 1cm.

Figure 6.

A: True tensile strength of silicone elastomer strips incorporating 5 %, 15 % and 30 % CA: SB previous to and after incubation (1 day, 1 week and 1 month) in PBS (pH 7.4, 37 °C). Results denote mean $\pm$ SD from 3 independently synthesized batches. **B:** Young's Modulus of silicone elastomer strips incorporating 5%, 15% and 30% CA: SB prior to and after incubation (1 day, 1 week and 1 month) in PBS (pH 7.4, 37°C). Results denote mean $\pm$ SD from 3 independently synthesized batches.

Table 1

Composition of silicone elastomer formulations.

Drug Powder		Formulation
Drug	Content (%)	Additives (%)
AM2/97	0.5 % w/w	CA:SB = 1:3, 0 % w/w
AM2/97	0.5 % w/w	CA:SB = 1:3, 5 % w/w
AM2/97	0.5 % w/w	CA:SB = 1:3, 15 % w/w
AM2/97	0.5 % w/w	CA:SB = 1:3, 30 % w/w
AM2/97	0.5 % w/w	CA:SB = 1:3, 40 % w/w
AM2/97	1 % w/w	CA:SB = 1:3, 30 % w/w

Table 2

Measurement of biological activity of the released inhibitors, using an enzyme linked sorbent assay. The results were expressed as a percentage activity of TG compared to positive control (without the inhibitor). Results denote mean $\pm$ SD from 3 independently synthesized batches.

Inhibitor (w/w)	% activity of TG		% reduction in activity of TG	
	1 %	0.5 %	1 %	0.5 %
time				
1 Day	9.12	16.06	90.88	83.94
1 week	6.55	12.06	93.45	87.94
2 week	54.34	57.90	45.66	42.10
3 week	46.16	50.23	53.84	49.77
4 week	78.30	82.20	21.70	17.80