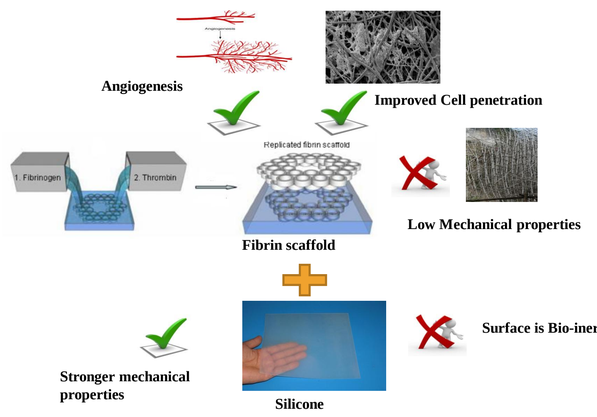
# Protein binding functionalization of plasma-derivatized silicone surfaces

**V Sharma[1, 4], K A Blackwood[1, 2], D Haddow[3], C Mason[4], J F Dye[1] and E García-Gareta[1]**

[1] RAFT Institute of Plastic Surgery, Mount Vernon Hospital, Northwood, Middx HA6 2RN, UK
[2]Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, QLD 4059, Australia
[3]Altrika Ltd, The Innovation Centre, 217 Portbellow, Sheffield S1 4DP, UK
[4] Advanced Centre for Biochemical Engineering, University College London, Gower Street, London, WC1E 6BT, UK

## Introduction

- A major concern related to large area skin wounds is loss of barrier function and current standard treatments (autologous split thickness grafts) have been associated with complications of scarring and lack of availability of donor sites.
- There is an increased need for biomaterials with stronger mechanical properties and better cell infiltration rates.




- Silicone (Si) surfaces can be made more biocompatible by introducing specific functional groups such as acrylic (AcA), allylamine, (AlA) through a relatively simple one-step coating process called plasma polymerization.
- This works aims to develop a composite scaffold with biological properties of fibrin and improved robust silicone backing.

## Aims

- Quantify fibrinogen (Fbg) absorption on derivatized Si surfaces, which has never been done before.
- Compare the binding strength (using surfactants) of Fbg and different derivatized Si surfaces.
- Cell attachment and proliferation on different derivatized Si using human dermal fibroblasts (HDFs) to confirm the biocompatibility of the protein-polymer complex.

## Methods

- Fbg was the model protein used and a novel technique based upon enzyme linked immune-sorbent assay (ELISA) was performed to understand protein binding and illustrate the binding strength with Si.
- This assay detected protein bound on to polymeric surfaces using specific antibody recognition system.

1. Fbg protein is added onto Si discs placed in a blocked well.
2. Blocking–buffer is added to block remaining protein-binding sites.
3. Fbg capturing primary antibody is added.
4. Anti-Fbg Alkaline phosphatase conjugate is added which binds to the primary antibody
5. PnPP substrate is added and converted by Alkaline phosphatase to detectable form.

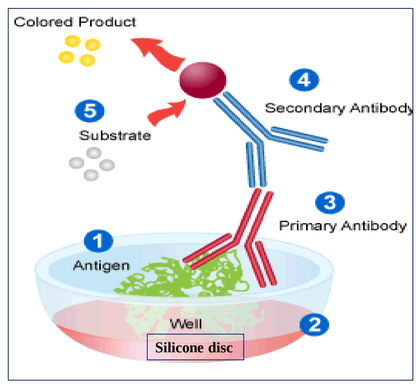



*Fig 1: Illustration of novel ELISA assay*

## Results

### 1. Plasma polymerization treatment roughens Si surfaces.

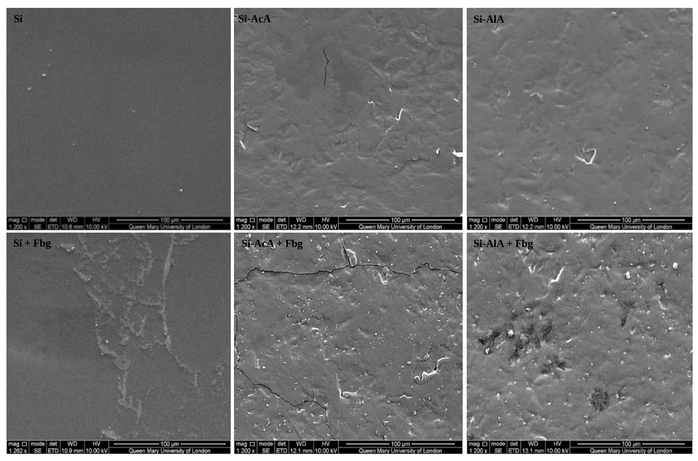



*Fig 2: SEM images of the different surfaces of the Si sheets (Si, Si-AcA. Si-AlA) used in this study coated and non-coated with fbg (150 µg/ml).*

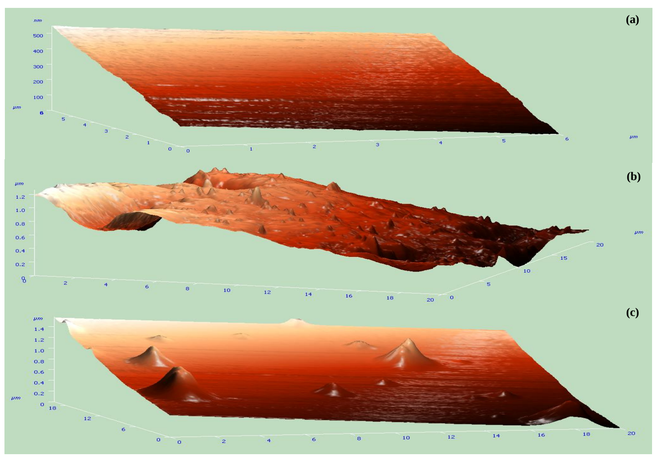



*Fig 3: Surface roughness images of different non-coated Si sheets visualized by atomic force microscopy (a) Si. (b) Si-AcA. (c) Si-AlA.*

### 2. Fbg binds with similar affinity to all Si surfaces.

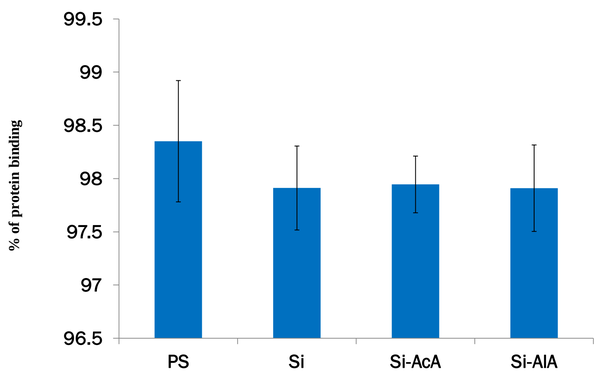



*Fig 4: Comparing the percentage of Fbg binding to different Si discs (Si, AcA & AlA) with polystyrene (PS) ELISA well to quantify bound protein. Results are the mean values ± SEM with n=3.*

### 3. Si-AcA has superior binding affinity to Fbg in compared to Si and Si-AlA.

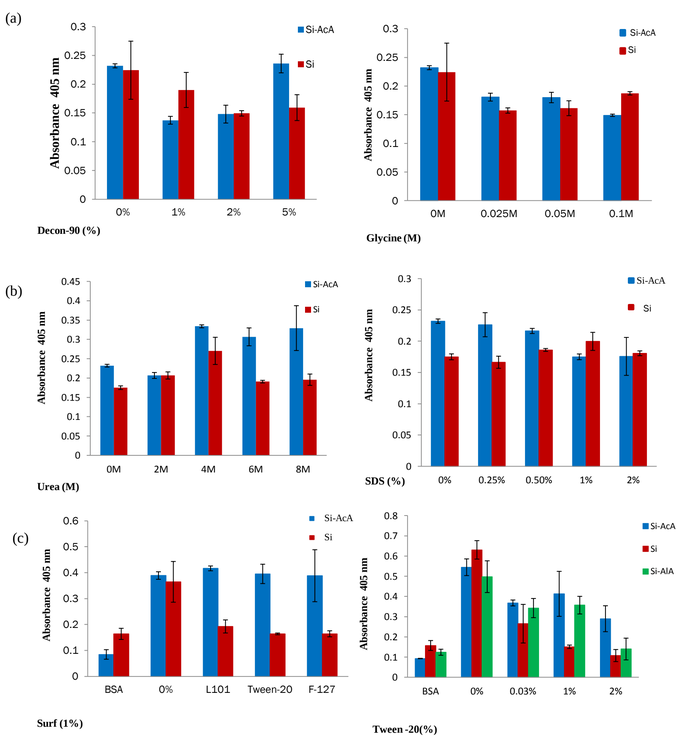



*Fig 5: ELISA assay to demonstrate binding strength of Fbg to derivatized Si (a) Decon-90(1%, 2%, & 5%) & Glycine (0.1 M, 0.025 M & 0.05 M) , (b) Urea (2 M, 4 M, 6 M & 8 M) & SDS (2 M, 4 M, 6 M & 8 M) and (c) Surfactants (L101,F-127 & Tween-20) at 1% &Tween-20 (0.03%, 1% & 2%). BSA (1%) was used as the negative control whereas the control wells (0% / 0 M) were not treated with these chemicals. Results are the mean values ± SEM with n=3.*

### 4. Si-AcA with / without Fbg showed higher metabolic activity than control (HDFs cultured on coverslips).

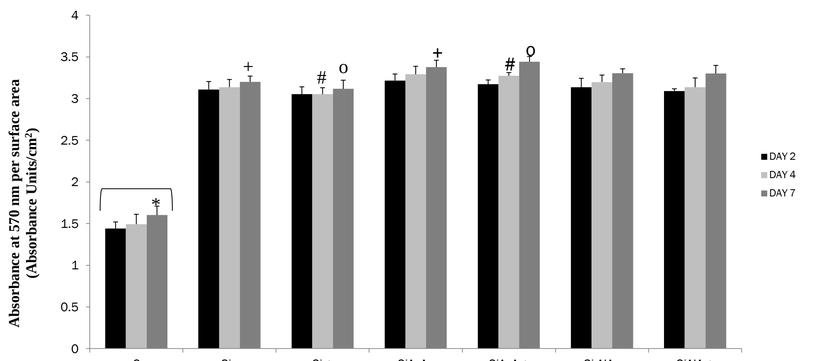
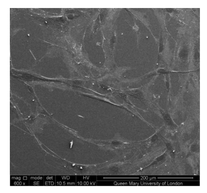
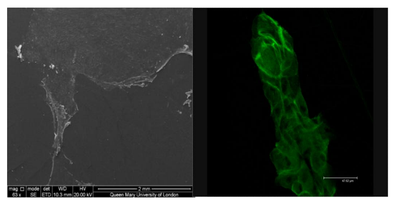



*Fig 6: Cell viability by Alamar Blue assay of HDFs cultured on coverslips (C), Si discs (Si), Si discs coated with fbg (Si+), Si-AcA discs (SiAcA-), Si-AcA discs coated with fbg (SiAcA+), Si-AlA discs (SiAlA-), Si-AcA discs coated with fbg (SiAcA+). C significantly lower at all time points than the different silicone surfaces (*p<0.001); significantly higher activity per surface area after 7 days of culture for Si-AcA discs compared with non derivatized Si discs (+p=0.047); higher activities per surface area for fbg coated Si-AcA samples compared to fbg coated non derivatized Si discs at days 4 (#p=0.012) and 7 (op=0.010) of culture.*

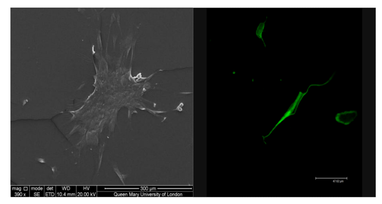
### 5. Si-AcA coated and non-coated with Fbg improves cell attachment in comparison to Si and Si-AlA.



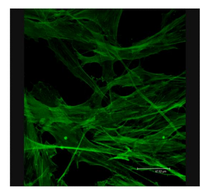
*SEM of HDFs cultured on coverslip as control.*



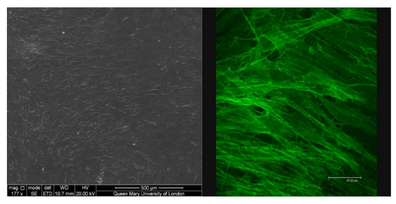
*SEM and confocal images of HDFs cultured on Si discs.*



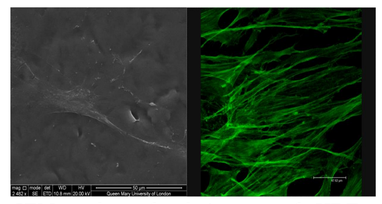
*SEM and confocal images of HDFs cultured on Si discs coated with Fbg.*



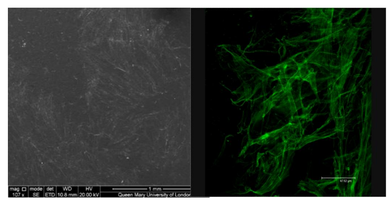
*Confocal images of HDFs cultured on coverslip as control.*



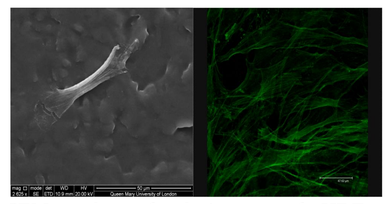
*SEM and confocal images of HDFs cultured on Si-AcA discs.*



*SEM and confocal images of HDFs cultured on Si-AcA discs coated with Fbg.*



*SEM and confocal images of HDFs cultured on Si-AlA discs.*



*SEM and confocal images of HDFs cultured on Si-AlA discs coated with Fbg.*

*Fig 7: SEM images along with confocal microscopy images of HDFs cultured on the different coated and non coated Si sheets stained with green phalloidin.*

## Conclusion

- A robust ELISA method to determine protein quantification and affinity from Si surfaces was successfully established.
- Fbg bound to Si sheets equally but on addition of surfactants it was proved Si-AcA had the highest binding strength followed by Si-AlA when compared to native Si.
- Cell culture analysis on the surface of these Si sheets in the presence of Fbg was also done as a confirmatory test to prove the AcA coated polymers have superior cell attachment and biocompatibility than native Si.
- This technique will be useful to improve current quantification assays of bound protein to various surfaces and also extend the application of Si as a medical device.