Methanol Washes Of Silicone Coatings Alter Activity Of Enzymes Involved In Barnacle Glue Polymerization

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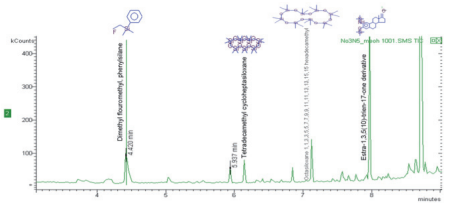
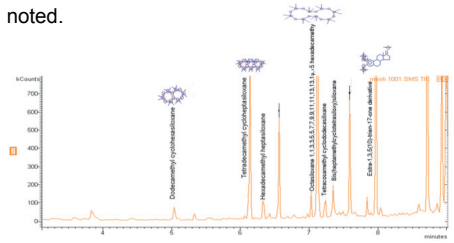
Introduction:

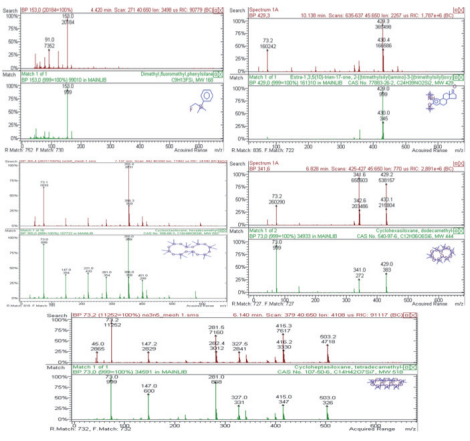
Silicone coatings are of interest since they are easy to clean of fouling, often for extended periods of time. There are a variety of hypotheses for why this is true, including physical properties such as modulus, and chemical interactions. We tested the hypothesis that, in part, clean-ability results from alterations in the way that natural adhesives polymerize. Our present model of barnacle glue polymerization includes a trypsin-like serine protease and a transglutaminase. The serine protease clips "pro" forms of structural proteins, enabling rearrangement and cross-linking by the transglutaminase. Thus adhesion and polymerization have at least two enzymatic steps and changes in enzymatic activity, either inhibition or promotion, could change the properties of the adhesive. We empirically tested the hypothesis if molecules leaching from silicone polymers impact activity of the serine protease and transglutaminase. We conducted brief washes of commercial silicone coatings with methanol, characterized the washes with GC mass spectrometry, and tested the impact of the silicone polymer washes on both native enzymes in polymerizing barnacle glue and on purified vertebrate trypsin and transglutaminase.



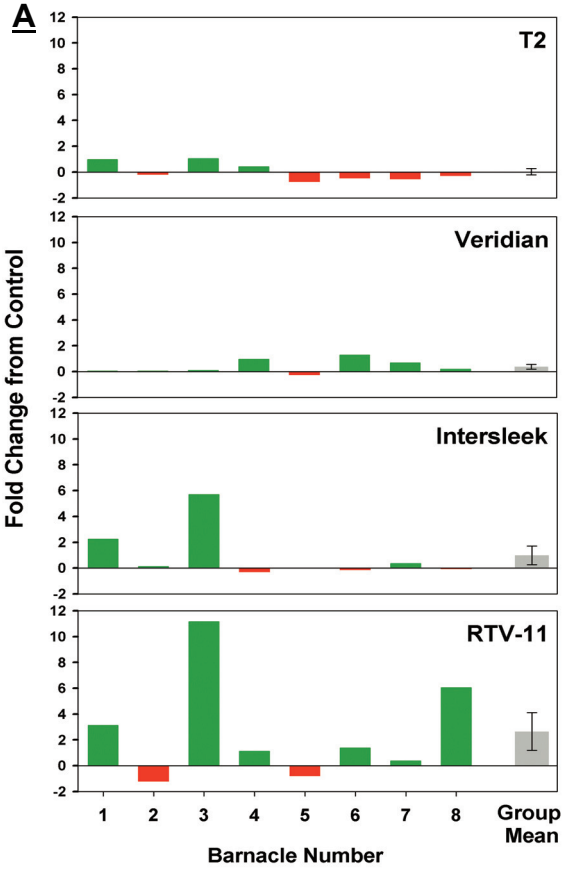
GC Trace with mass spectrometry identification for **International Paints Veridian® silicone**, extracted for 30 sec with 100% methanol. Name and structure (if available) of extracted molecules identified in the NIST database are noted.

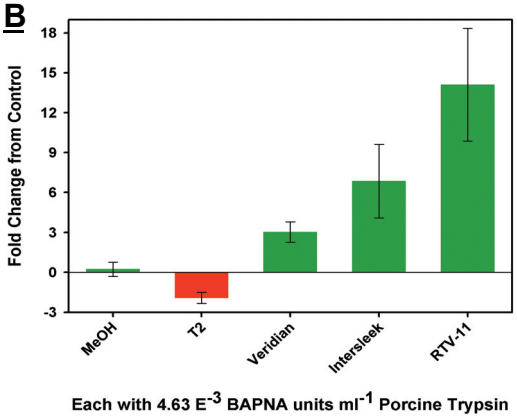


GC Trace with mass spectrometry identification for **Dow Corning Silastic T2® silicone**, extracted for 30 sec with 100% methanol. Name and structure (if available) of extracted molecules identified in the NIST database are noted.



Mass spec traces for GC peaks shown above with matching NIST mass spectral library traces (green)





Each with 4.63 E^{-3} BAPNA units ml^{-1} Porcine Trypsin

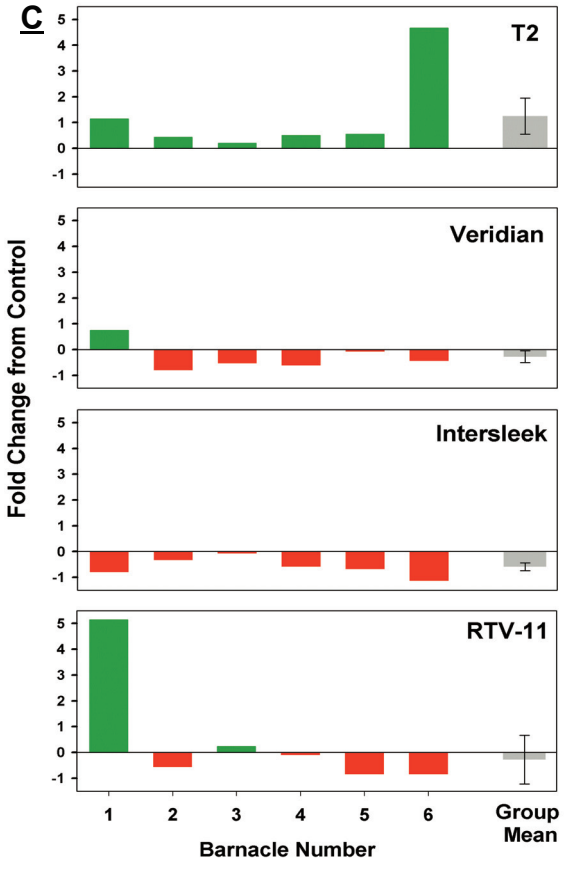
***A - D:* The effect of silicone extracts on enzyme activity.** Silicones were extracted with 600 µl 100% MeOH for 30 sec, dried, then resuspended in 10 µl 100% MeOH for assays. Promotion is shown in green, inhibition in red. Error bars are standard error.

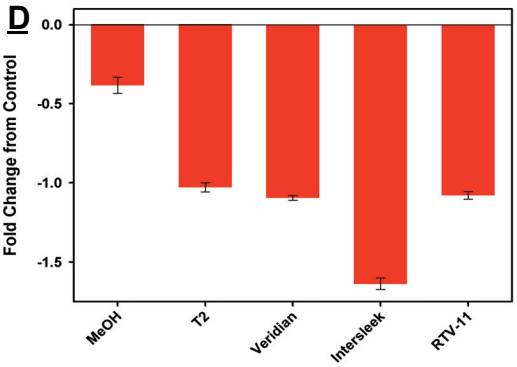
***A:* Barnacle glue trypsin**. Each individual barnacle was tested with all four silicones. Data, expressed as fold change from control (barnacle glue with 10 µl MeOH only), is show for each individual and as a group mean for each silicone. 6 µl unpolymerized glue was incubated with 800 µl BAPNA (1 mM) at 37°, pH 8.0, centrifuged at 9000 rpm for 10 min and absorbance was read at 405nm.

***B:* Purified porcine trypsin**. Assays conducted as described above using commercially available, purified porcine trypsin. Data, expressed as fold change from control (trypsin with 10 µl water only).

***C:* Barnacle transglutaminase**. Each individual barnacle was tested with all four silicones. Data, expressed as fold change from control (barnacle glue with 10 µl MeOH only), is show for each individual and as a group mean for each silicone. Assays were conducted using a TGase kit (Sigma #CS1070), with 1 µl unpolymerized glue per well.

***D:* Purified guinea pig TGase**. Assays conducted as described above using commercially available, purified guinea pig TGase. Data, expressed as fold change from control (TGase with 10 µl water only).





Each with 2 milliunits ml^{-1} Guinea Pig TGase

Conclusions

1. GC coupled mass spec allowed identification of specific molecules released from silicone polymers.
2. The impact of silicone polymer washes on serine-like protease activity in polymerizing barnacle glue is dependent upon the individual barnacle.
3. We were surprised to observe multifold stimulation of activity by the interaction of specific polymer washes with specific barnacle glues and with purified porcine trypsin.
4. The impact of silicone polymer washes was variable and not as dramatic on transglutaminase activity of individual barnacles. Activity in some individuals was stimulated, while others inhibited.
5. Guinea pig transglutaminase was inhibited by all silicone washes.
6. Altering activity of trypsin and transglutaminase should alter the effectiveness of adhesive polymerization and explains, in part, why silicones are easy clean surfaces.

Acknowledgements

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