Modification of fibrin structure with Pure Hypochlorous Acid-Based Wound Solution

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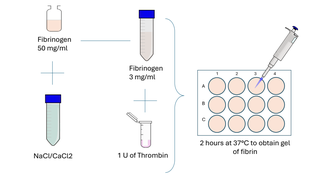
Introduction Delayed wound healing represents a major clinical challenge and imposes a substantial burden on healthcare systems. A major impediment to effective healing is the presence of **abundant slough**, whose removal is a critical therapeutic step. Conventional **debridement** procedures are available but frequently associated with patient discomfort. **VASHE®, a stabilized hypochlorous acid (HOCl) solution**, has emerged as an alternative for wound cleansing and slough removal.

The objective of this study was to evaluate the effect of HOCl solution on a fibrin gel model mimicking slough composition, at various time points.

Methods

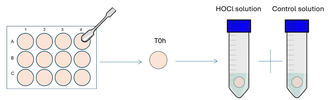
Fibrin gel formation in 12-well plates

Fibrinogen is polymerized into insoluble fibrin by thrombin. A mix of fibrinogen and thrombin (2 mL) is deposited in 12-well plates and incubated for 2 hours at 37°C before the fibrin gel is ready to be used.
Fibrin polymerization is assessed by spectrophotometry at 450 nm using VANTAstar.



Vashe effect on fibrin gel

To evaluate Vashe's effect on fibrin gel, fibrin gel is extracted from the well and immersed in 20 mL of pure HOCl solution or control solution for 3 and 24 hours.



Viscoelastic Analysis

Fibrin viscoelastic properties were characterized by oscillatory rheological measurements using a parallel-plate geometry. Tests were performed at 35 °C under a constant strain of 1% and a frequency of 1 Hz. Viscoelastic properties were evaluated before and after exposure to HOCl solution.

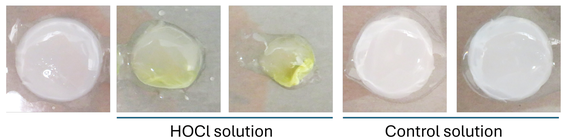
Mass Spectrometry Analysis

Fibrin gels are grinded in bead tubes.
A lysis buffer is added, followed by protein reduction/alkylation. Peptides are digested by a Trypsin/LysC enzyme mixture, then purified by column washing. The iST 96x kit (PréOmics) is used for these steps.

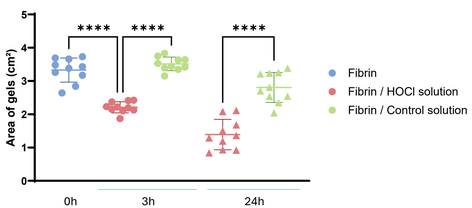
Peptides are resuspended at a concentration of 1 g/L for injection into LC using an Acquity UPLC HSS T3 column before being analyzed by mass spectrometry. Analysis is performed using a Vanquish pump (Thermo Fisher) and an OrbiTrap Exploris 240 (Thermo Fisher).
Results are reprocessed using Proteome Discoverer software.

1. Marked structural modification of fibrin Gel

1A. Macroscopic modification of fibrin gel

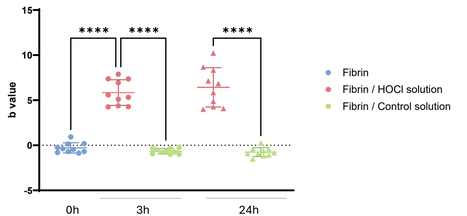


1B. Shrinkage of fibrin gel



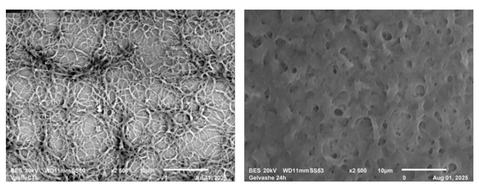
Area of the gel in cm^2 measured by ImageJ
ANOVA test **** p<0.0001

1C. Increase color alteration of fibrin gel



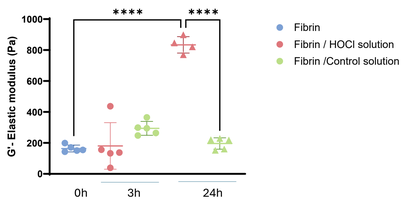
Colorimetric analysis of axis b (yellow-blue) using CM-26dG Portable Spectrocolorimeter
ANOVA test **** p<0.0001

2. Contraction of fibrin network



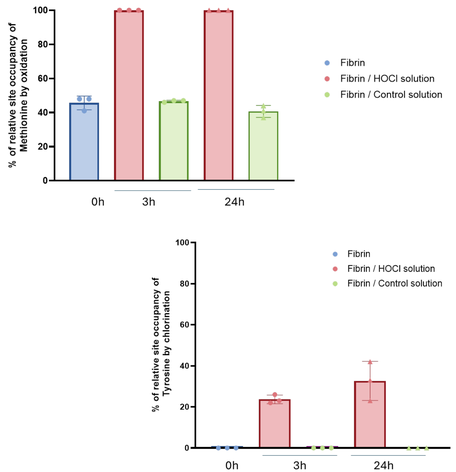
SEM observation of fibrin gel network - 6 µm paraffin sections)

3. Increase stiffness of fibrin gel



Elastic modulus of fibrin gel using a parallel-plate geometry rheometer
ANOVA test **** p<0.0001

4. HOCl solution induces oxidation and chlorination



Oxidation and Chlorination % of relative site occupancy of Methionine and Tyrosine using Mass Spectrometry analysis

Conclusion VASHE®, pure hypochlorous acid-based solution, induces marked structural modification of fibrin gel after 3h of contact. Increase in oxidation and chlorination of fibrinogen peptides of the fibrin may explain the shrinkage of the gel (due to possible disulfite bridge formation) and the yellow color appearing (in link with the increase chlorination over time). These structural modifications may alter fibrin conformation and adherence leading to a better slough debridment in patients.

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References :
1. Vissers MCM et al. Myeloperoxidase-derived oxidants HOCl and chloramines. In: Hydrogen Peroxide Metabolism in Health and Disease. CRC Press, 2017.
2. C. L. Hawkins, Hypochlorite-induced oxidation of amino acids, peptides and proteins, Amino Acids, Volume 25 p 259-274, 2003