Pure Hypochlorous Acid-Based Wound Solution prevents fibrin formation

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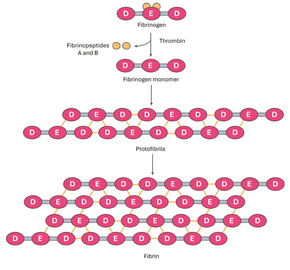
Introduction

Delayed wound healing poses a major clinical challenge and places a significant burden on healthcare systems. A key impediment is the accumulation of slough, which must be removed for effective healing. Conventional debridement methods are often associated with patient discomfort. **VASHE®, a stabilized hypochlorous acid (HOCl) solution**, has emerged as a potential alternative for wound cleansing and slough removal.

The objective of this study was to evaluate the effect of HOCl solution on *in vitro* fibrin formation.

Methods

Fibrin gel formation

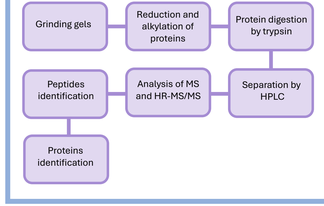


Fibrin polymerization is assessed by spectrophotometry at 450 nm using a multimode microplate reader (VANTAstar; BMG Labtech).

HOCl solution is added to Fibrinogen or/and/or Thrombin (so-called Fibrinogen/HOCl solution or thrombin /HOCl solution) before polymerization to evaluate its impact.

Proteomic analysis protocol steps using HPLC-MS to process fibrin gel

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. HOCl prevents the formation of the fibrin gel

Macroscopical observations of fibrin gels

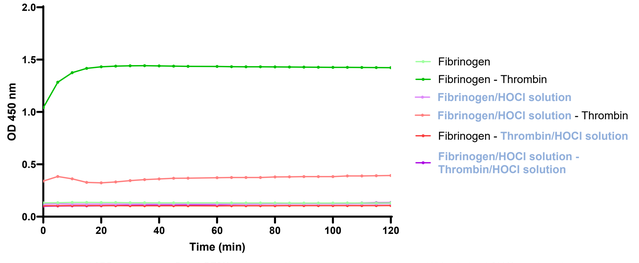
Fibrinogen - thrombin

Fibrinogen/HOCl solution - thrombin

Fibrinogen - thrombin /HOCl solution

Fibrinogen/HOCl solution - thrombin/HOCl solution

Modification of polymerization curve during fibrin formation

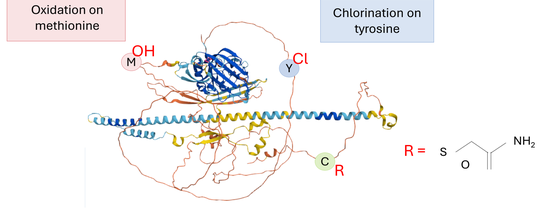


Absorbance analysis (450 nm) during 2h at 37°C using multi-mode microplate reader VANTAstar (BMG Labtech).

Clot turbidity increases during the first 10 minutes and then stabilizes.
In the presence of HOCl solution, curves remain flat except for the Fibrinogen/HOCl–Thrombin condition, likely due to an excess of fibrinogen over HOCl.
Consistently, no clot is observed macroscopically.

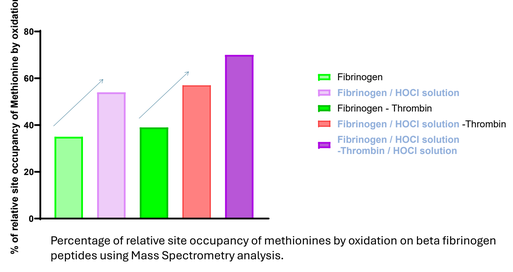
2. HOCl solution induces oxidation of methionines of fibrinogen

2A. Chemical modification that could occured on fibrinogen



Three-dimensional structure of fibrinogen protein

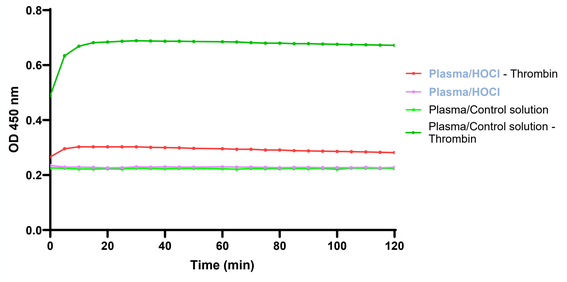
2B. Oxidation of fibrinogen impaires fibrin gel formation



Percentage of relative site occupancy of methionines by oxidation on beta fibrinogen peptides using Mass Spectrometry analysis.

The low concentration of thrombin makes its analysis by mass spectrometry impossible; therefore, only fibrinogen is analyzed.
An increase in methionine oxidation is consistently observed after adding HOCl to fibrinogen, either alone or during its polymerization into fibrin gel.

3. HOCl prevents polymerization of plasma into fibrin



Absorbance analysis (450 nm) during 2h at 37°C using multi-mode microplate reader VANTAstar (BMG Labtech).

In plasma, thrombin induces fibrinogen polymerization into fibrin. Addition of HOCl completely suppresses clot formation, with no increase in absorbance, indicating that HOCl prevents fibrin formation even in a complex protein matrix.

Conclusion

HOCl solution disrupts fibrinogenesis by altering fibrinogen and reducing thrombin activity, which prevents fibrinogen from polymerizing into insoluble fibrin. This mechanism could explain how HOCL reduces slough by preventing new fibrin formation in the wounds.
Consequently, VASHE® is considered a valuable therapeutic adjunct in wound management, particularly during the debridement phase.

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Reference:
1. Vissers MCM *et al*. Myeloperoxidase-derived oxidants HOCl and chloramines. In: Hydrogen Peroxide Metabolism in Health and Disease. CRC Press, 2017.