

Imprint: Novel sampling and cultivation method maintaining 2D organization of microbes

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Objective

To construct a reliable method for cultivating surface microbes from chronic ulcers while maintaining 2D spatial organization.

The method is called “Imprint”.

Background

Chronic ulcers affect up to 1% of the population worldwide. Biofilm infections negatively affect healing.

Mapping the spatial organization of microbes in chronic ulcers could aid the understanding of colonization/infection and improve treatment.

The current gold standard for cultivation involves swabbing a 1cm² surface area (the Levine technique).

Swabbing techniques offer little to no information about the 2D spatial organization of the bacteria. However, previous research on biopsies have shown that biofilms are heterogeneously distributed within chronic ulcers.

We aimed to develop a method that simultaneously cultured bacteria while recording spatial information about their organization in the ulcer.

Method

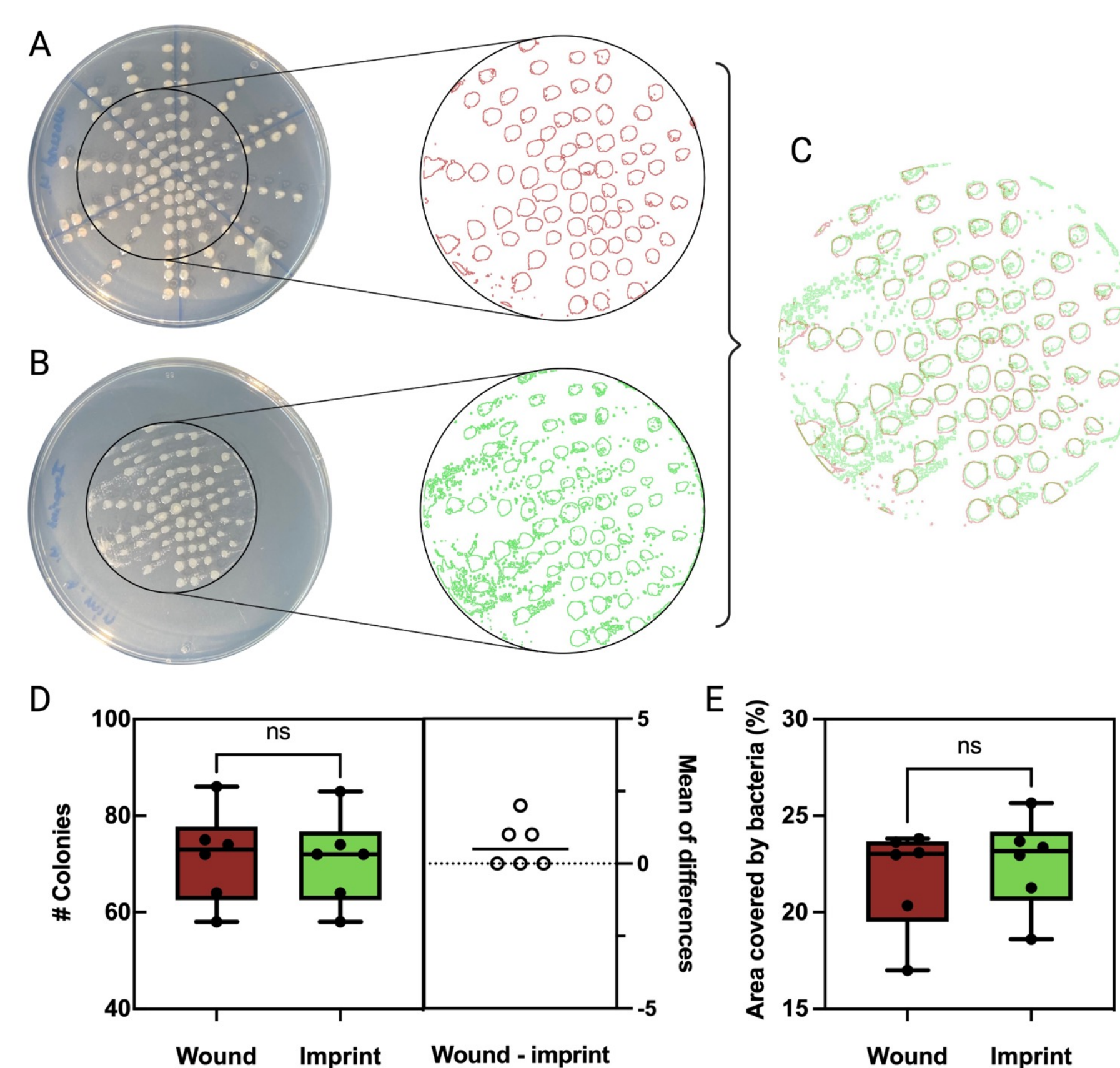
Filters were tested *in vitro* for their ability to transfer bacteria and maintain 2D geography by transferring colonies in distinct patterns. The filter that performed the best was tested *in vivo* on 20 patients suffering from chronic ulcers of the lower limbs to gain insight into clinical feasibility and the ability of Imprint to culture microorganisms *in vivo*.

Results

1) *In vitro*

Testing concluded that the nylon 5 µm filter (GVS North America) was able to successfully transfer bacteria and maintain patterns for both single and mixed species. Testing was done for *S. aureus*, *P. aeruginosa*, *E. faecalis*, *E. coli* and *C. striatum*.

All strains were previously isolated from patients with chronic ulcers.

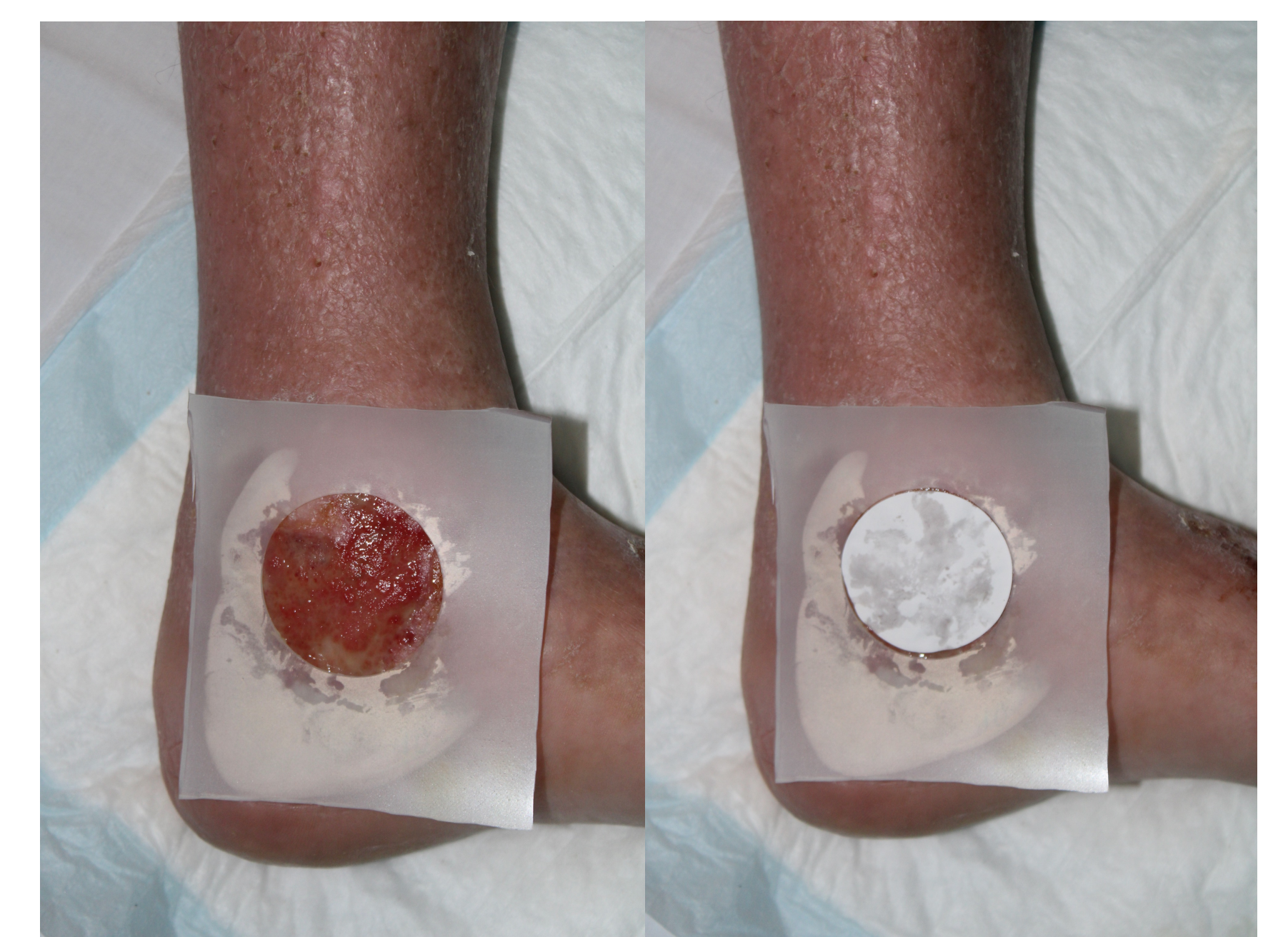


In vitro experiment examining the ability of Imprint to transfer bacteria from one culture plate to another while maintaining 2D organization.

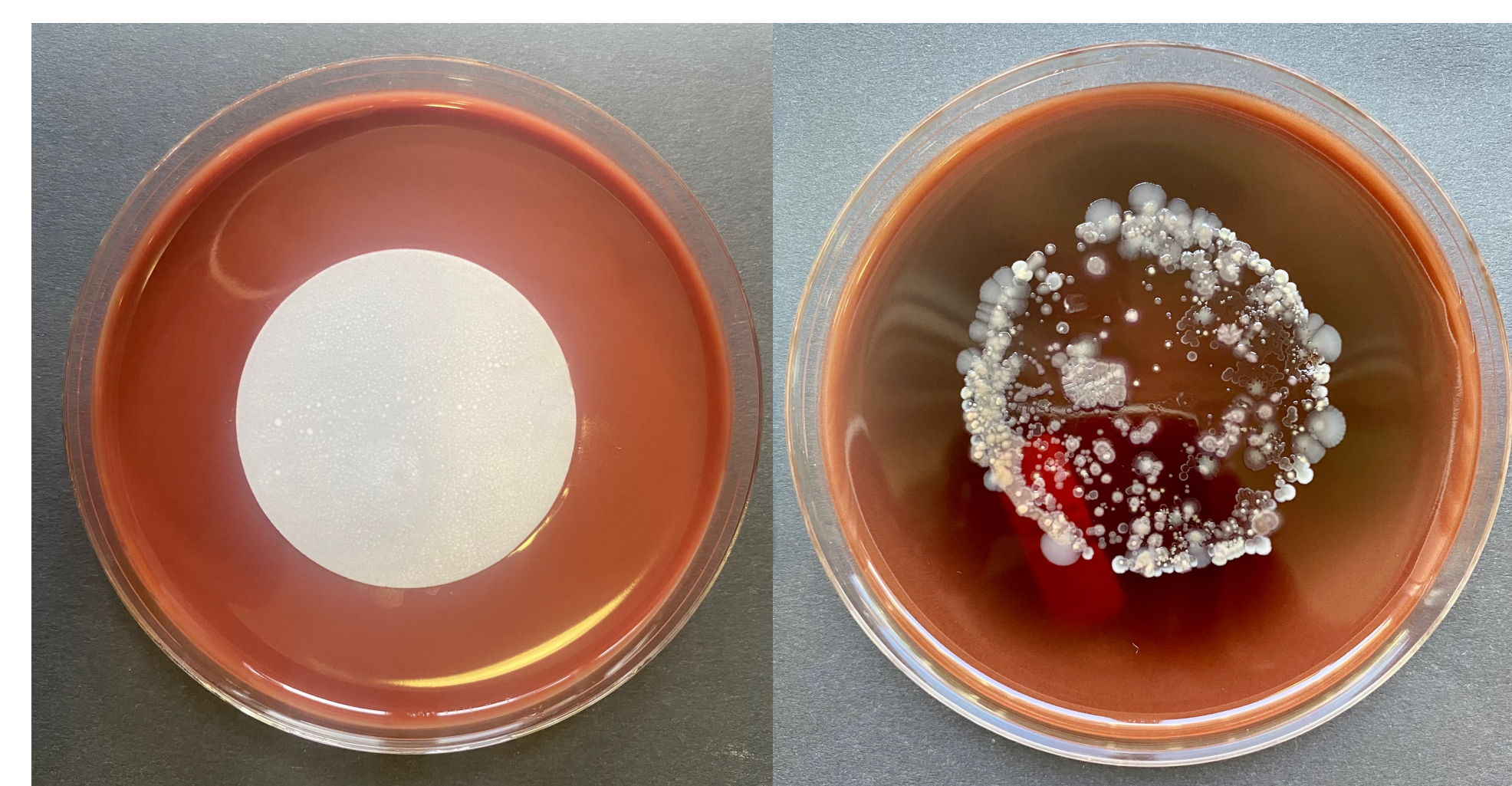
- Single colonies of bacteria were plated in a pattern to simulate an ulcer. Immediately after placement of colonies a filter was applied to the “ulcer plate”. After 30-60 seconds the filter was removed and the “ulcer plate” was left to incubate for 48 hours at room temperature. The photo shows the “ulcer plate” after 48 hours of incubation. The area where the filter was applied is outlined in the color red.
- The filter was transferred and placed on a clean plate named “imprint plate”. The filter was left on the plate for 3 hours followed by removal and the filter was discarded. The photo shows the “imprint plate” after 48 hours of incubation at room temperature. The resulting imprint is outlined in the color green.
- Comparison of the overlap between the “imprint plate” and the “ulcer plate” documenting maintenance of the original pattern.
- Comparison between number of bacterial colonies from “ulcer plate” and “imprint plate” showing non-significant changes between the two.
- Calculated area covered by bacteria on “ulcer plate” and “imprint plate” showing non-significant changes between the two.

2) *In vivo*

Testing was performed on 20 patients, all suffering from chronic ulcers on the lower limbs. Imprint was successful in culturing microorganisms from patients and easy to perform in the clinic.



Clinical photo of an Imprint being performed on a chronic lower limb ulcer. Ulcer with a sterile silicone sheet making it possible to perform the Imprint from a defined area (left). The filter is applied for 30-60 seconds (right) and transferred to a plate creating the Imprint.



Imprint plate from a chronic ulcer before (left) and after removal of the filter followed by incubation (right). The filter was removed from the imprint plate after 3 hours and the plate was incubated at room temperature for 48 hours.

Conclusion

- Imprint is a novel method for culturing bacteria from chronic ulcers.
- Imprint maintains 2D spatial organization *in vitro* for common wound pathogens.
- Imprint is user-friendly and easy to use in the clinic and successful in culturing microorganisms from patients suffering from chronic ulcers.



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