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**Title: Paper-based detection device for chronic wound monitoring**

**Reviewer #1**

This manuscript summarizes a very preliminary work on the evaluation of a paper-based device for detecting the level of human neutrophil elastase (HNE) in wound exudates.

**Comment 1:** There is very little information about the device. It is not clear the sensitivity and reproducibility of the device. The potential role of HNE in chronic wounds has yet to be determined.

**RESPONSE:** Thank you for your question. The sensitivity of our device in a wound fluid system is approximately 0.631  $\mu\text{g/mL}$  (DOI: 10.1039/d0lc00062k). We have also tested device reproducibility. We spiked three different concentrations of HNE (5  $\mu\text{g/mL}$ , 10  $\mu\text{g/mL}$ , and 25  $\mu\text{g/mL}$ ) using a tear system and obtained intra- and inter-assay coefficients of variation (CV) as follows: A) intra-assay CV (%) were 6.57 for 25  $\mu\text{g/mL}$ , 7.25 for 10  $\mu\text{g/mL}$ , and 6.66 for 5  $\mu\text{g/mL}$ , and B) inter-assay CV was 12.89 for 25  $\mu\text{g/mL}$ , 14.34 for 10  $\mu\text{g/mL}$ , and 16.15 for 5  $\mu\text{g/mL}$  (DOI: 10.1039/d0lc00062k).

**Comment 2:** The results present in Table 1 show wide variations between different patients and wounds. However, it is not clear how HNE elevation can be used for the diagnosis and care of chronic wounds. Without the above information, it is important to assess the potential impact of the device.

**RESPONSE:** Thank you for your question. Studies have shown that the level of HNE is higher in chronic wounds compared to healing wounds, (G. S. Schultz, G. Ladwig and A. Wysocki, *World Wide Wounds*, 2005, 2005, 1–18; Y. Bai, H. Wang and Q. Zhao, *Anal. Bioanal. Chem.*, 2017, 409, 6843–6849) which indicates that wound healing status can be monitored by detecting the level of HNE in wound exudate. (DOI: 10.1039/d0lc00062k)

**Reviewer #2**

This paper is very brief and lacks detail with regards to the context, experimental design and results. It needs substantial revisions and should not be published in its current form.

*The abstract requires more detail:*

**Comment 1:** 4: at a certain recovery stage: specific?

**RESPONSE:** The definition of chronic wounds are wounds that cannot be healed within three months. These wounds do not proceed toward the next healing stage. There are four stages associated with chronic wound healing: coagulation, inflammation, proliferation, and maturation. According to the studies referenced, chronic wounds are often stuck at the inflammation stage. (DOI: 10.2147/cia.s4726)

**Comment 2:** 6-8: rather vague information about elderly age group and chronic wound infection.

**RESPONSE:** As we mentioned in sentences 9 to 15 of our Introduction section, a significantly higher incidence of chronic wounds was found in the geriatric population compared to younger populations. Because it is difficult for the elderly to engage in frequent hospital visits, an efficient, accurate, and easy-to-use diagnostic tool for chronic wound monitoring is warranted.

**Comment 3:** 10-12: why is it useful for the elderly? Surely useful for all chronic wounds.

**RESPONSE:** Thank you for your question. As we mentioned in our article, due to the increased prevalence of comorbid conditions among the elderly (i.e., diabetes) a higher incidence of chronic wounds was found in the geriatric population compared to younger populations.

**Comment 4:** Does this test assess infection in chronic wounds?

**RESPONSE:** Thank you for your question. In this study, we did not include an infection test for chronic wounds, we primarily focused on the detection of HNE level among chronic wounds.

**Comment 5:** Clearer context is need for choice of demographic and biomarker.

**RESPONSE:** Thank you for your comment. As mentioned in sentence 4 of our Method section, HNE plays a vital role during wound healing phases and can thus be used as a prognostic factor for wound

status assessment. Furthermore, the reason why we chose N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide as our substrate is because it is a frequently used chromogenic substrate for HNE detection. (J. Edwards, S. Caston-Pierre, A. Bopp and W. Goynes, *J. Pept. Res.*, 2005, 66, 160–168.)

*Introduction:*

**Comment 6:** 37-38: annual costs globally? Detail needed

**RESPONSE:** According to one study, in the US alone, the cost for treating chronic wounds is approximately 5 to 10 billion US dollars each year. (Werdin, F., Tennenhaus, M., Schaller, H. E., & Rennekampff, H. O. (2009). Evidence-based management strategies for treatment of chronic wounds. *Eplasty*, 9.)

**Comment 7:** The introduction overall is rather brief – some rationale is given for the need of this device and the demographic, but this should be expanded upon and facts checked for clarity.

**RESPONSE:** Thank you for your comment. We have added some text supporting the need for our device (see highlighted text, sentences 17 to 22 of our Introduction section), and the rationale behind our biomarker selection. Details regarding device fabrication can be found in the first paragraph of our Methods section.

*Methods:*

**Comment 8:** 64-68: this type of information should be in the discussion

**RESPONSE:** Thank you for your comment. We have moved this paragraph from the Methods section to the Discussion section (see highlighted text in the first paragraph of our Discussion section).

**Comment 9:** The information here does not describe the actual methods employed i.e. it is not possible to reproduce the study based on these methods. The style is more like a discussion/introduction

**RESPONSE:** Thank you for your comment. We have elaborated further on the production process and the application of our device in this article: DOI: 10.1039/d0lc00062k, which includes details regarding all experimental methods, and we have added some text regarding device fabrication in our Methods section (see highlighted text, sentences 12 to 16 of our Method section).

*Results:*

**Comment 10:** no detail is given in the results to allow for critical appraisal of the findings.

**RESPONSE:** Thank you for your comment. We have added several sentences describing the meaning of our findings in our Results section (see highlighted text, sentences 12 to 15 of our Results section).

*Discussion:*

**Comment 11:** introduces information not included in the method or results, and is not an appraisal of the findings in the broader context of the field.

**RESPONSE:** Thank you for your comment. We have added some descriptive content regarding device fabrication (see highlighted text, sentences 12 to 16 of our Methods section), and the LODs of the device in buffer and tear systems (see highlighted text, sentences 1 to 3 of our Results section).