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# Monitoring of the Evolution of Human Chronic Wounds Using a Ninhydrin-Based Sensory Polymer and a Smartphone †

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**Abstract:** The healing processes in cutaneous wounds, i.e., chronic wounds, represent a health problem affecting 1-2% of the population. The evaluation of these wounds is mainly based on subjective parameters, although there is a medical consensus on protease activity as the best marker for healing disorders. Here we show the correlation of the amino acid concentration on chronic wounds and their evolution, and the development of a test kit to straightforwardly determine this evolution. We analyzed with the kit the amino acid concentration of human chronic wounds of 34 patients, and we mathematically demonstrate that there is a correlation with the amino acid concentration, related to the protease activity and the evolution of the wound's diagnoses.

**Keywords:** monitoring human chronic wounds; polymer chemosensor; smartphone

## 1. Introduction

Chronic wounds are one of the many problems facing the medical community. These types of injuries are very common, but they are very difficult to diagnose. Currently, doctors assess wounds after a visual analysis. The problem with this assessment is that it varies from one doctor to another, and until after a few days, it is not known if the wound is actually healing correctly or becoming a chronic wound. These lesions are associated with imbalances between the degradation/regeneration processes of cell membranes derived from the activity of metalloproteases. Under normal conditions, these enzymes degrade damaged tissues so that healthy ones can replace them. When their activity gets out of control, they attack healthy and damaged tissues indistinctly, thus preventing wound healing. Therefore, the enzymatic activity of metalloproteases is the most appropriate biomarker for evaluating the state of a wound [1]. In this work, it is proposed to indirectly measure the enzymatic activity of proteases by determining the concentration of amino acids derived from the degradation of membrane proteins. That is to say, higher enzymatic activity, a higher concentration of amino acids. To carry out this measure, it has been designed a colorimetric sensory polymeric film based on ninhydrin [2], which changes its color when in contact with amino acids. This color change will be analyzed through the RGB parameters of a digital photo taken with a smartphone.

## 2. Methods

### *2.1. Synthesis*

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For the synthesis of a polymer with receptor units based on ninhydrin, we prepared a monomer based on ninhydrin whose subsequent radical copolymerization of two main comonomers, one hydrophilic (vinylpyrrolidone, VP) and the other hydrophobic (methylmethacrylate, MMA), resulted in a functional polymer film that was transformed into the sensory polymer containing ninhydrin-based receptors by direct solid-phase synthesis. (see Figure 1). This methodology is more profitable and ecological than the traditional one, since it reduces solvents' use and the time required.

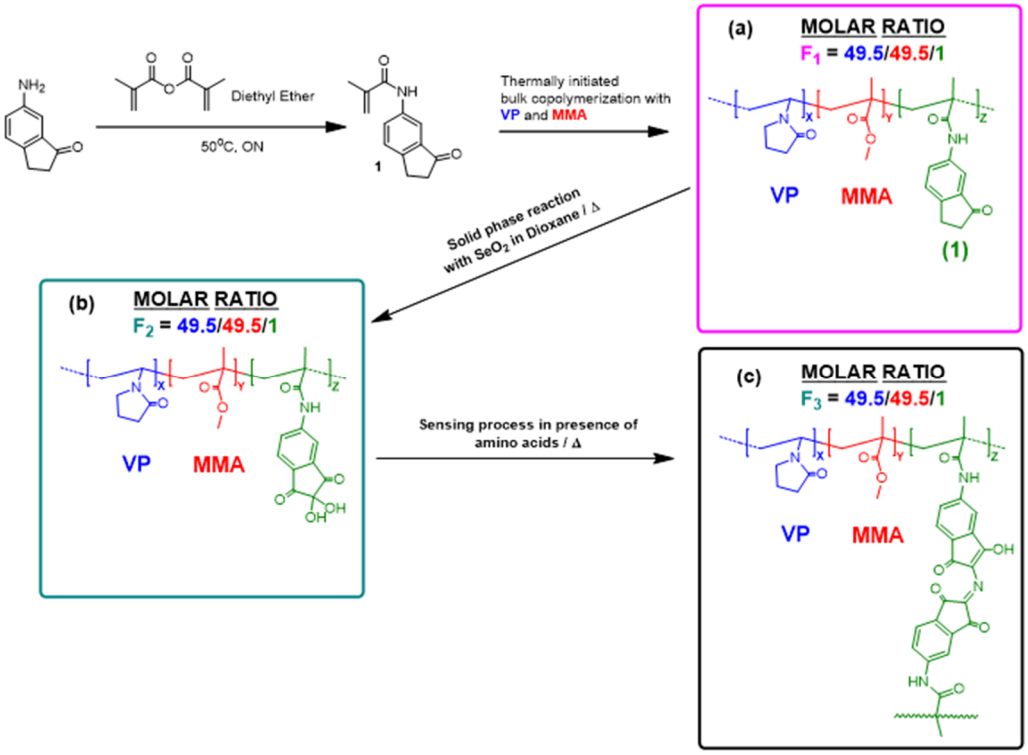


**Figure 1.** Synthetic route for the sensory monomer 1; (**a**) Sensory polymer before solid-phase reaction; (**b**) Sensory polymer after solid-phase reaction; (**c**) Sensory polymer reaction in the presence of amino acids.

### *2.2. Material Characterization*

The material was thermally characterized by TGA and DSC ($T_5$, $T_{10}$, $Tg$: 296, 355, 206 °C), and mechanically evaluated (Young's modulus: 31 MPa). Additionally, pH stability assays, solid-state NMR-$^{13}$C, FT-IR, and RAMAN experiments were performed.

## 3. Results and Discussion

An interference study was carried out, and its sensory capacity was tested with synthetic solutions mimicking epidermis (see **Figure 2**), collagen, and elastin. The results were contrasted with a reference method for quantifying amino acids (Nielsen method)[3].

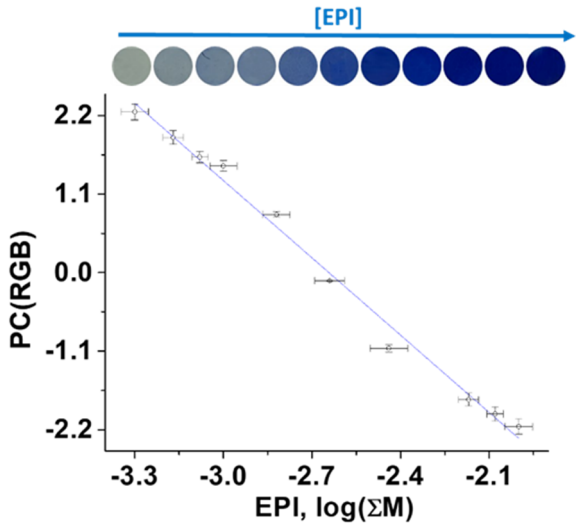


**Figure 2.** Titration of F2 discs with solution mimicking epidermis (EPI) was carried out by studying the RGB digital color parameters (RGB_method). Discs of 8 mm diameter of sensory material were dipped in pH 4.66 buffered solutions of EPI, with a sum of concentrations of all amino concentrations ranging from $5x10^{-4}$ to $1x10^{-2}$ M (ΣM). After reaction at 100ºC for 60 min, the discs were washed several times with water, and photographed for the extraction of RGB data, which were simplified to a single variable (principal component, PC) through a multivariate analysis.

Finally, two proofs of concept were carried out, with a food matrix and 34 chronic wound samples from 34 patients.

**Table 2.** Results obtained by the diagnostic methods (discriminant analysis, **DA**, logistic regression, **LR**, and support vector machine, **SVM**) using both reference method and RGB_method as explanatory variables (absorbance at 330 nm and RGB parameters respectively).

| Experimental method | Medical parameter | Cases | | Analysis method | | |
|---|---|---|---|---|---|---|
| | | No | Yes | DA | LR | SVM |
| **Reference method** | Infectious aspect | 26 | 9 | 16 **(Sc)** | 26 | 26 |
| | | | | *0.4571* ***(rat)*** | *0.7429* | *0.7429* |
| | Bad evolution | 21 | 14 | 20 | **24** | 21 |
| | | | | *0.5714* | *0.6857* | *0.6* |
| | Necrosis | 26 | 9 | 24 | 25 | 26 |
| | | | | *0.6857* | *0.7143* | *0.7429* |
| | Ischemia | 28 | 7 | 24 | 27 | 28 |
| | | | | *0.6857* | *0.7714* | *0.8* |
| **RGB_method** | Infectious aspect | 25 | 9 | 19 | 23 | 25 |
| | | | | *0.5588* | *0.6765* | *0.7353* |
| | Bad evolution | 21 | 13 | 19 | **22** | 21 |
| | | | | *0.5588* | *0.6471* | *0.6176* |
| | Necrosis | 25 | 9 | 21 | **31** | 25 |
| | | | | *0.6176* | *0.9118* | *0.7353* |
| | Ischemia | 27 | 7 | 22 | 26 | 27 |
| | | | | *0.6471* | *0.7647* | *0.7941* |

## 4. Conclusions

We have developed a new method and methodology for controlling and diagnosing chronic wounds based on pictures taken to discs cut from sensory films, which change their color upon entering into contact with amino acids. The sensory polymeric material

is cheap, the experimental procedure is simple, expensive equipment isn't needed, and can be straightforward carried out by untrained personnel by taking a photograph with a smartphone to the sensory material after immersing in the exudate. Moreover, we have demonstrated that the state/evolution of the wound correlates with the concentration of amino acids. The reference method (Nielsen method)[3] and RGB_method have shown good results in wound diagnosis. Mainly, in the case of bad evolution, the results are fascinating. Even, the RGB values show to have a diagnostic capability of chronic wounds similar to the reference method, and better in the case of necrosis. We have used linear models, for data treatment and predictions. However, more sophisticated models could significantly improve the quality of the results and be integrated into an easy-to-use software or smartphone app.

## 5. Patents

This work resulted in a patent: "Guembe García, M., Vallejos Calzada, S., García Pérez, J. M., García García, F., Ibeas Cortés, S., & Yagüe Fernández, P. (2020). Copolímeros de estructura derivada de la ninhidrina, películas o membranas obtenidas a partir de los mismos y su utilización. N⁰ P202030077. Priority country: Spain. Ownership: University of Burgos".

**Author Contributions:** Conceptualization, M.G.G., V.S.G., N.M.R., F.C.G., J.M.G., S.V.; methodology, M.G.G., V.S.G., N.M.R., S.I., J.A.R., F.C.G., J.R., S.C., J.M.G., S.V.; software, M.G.G., S.I., J.A.R., F.C.G., J.P., S.C., J.M.G., S.V.; validation, M.G.G., V.S.G., N.M.R., S.I., J.A.R., F.C.G., J.P., S.C, J.M.G., S.V.; formal analysis, M.G.G., S.I., J.A.R., F.C.G., J.P., S.C., J.M.G., S.V.; investigation, M.G.G., V.S.G., N.M.R., S.I., J.A.R., F.C.G., J.P., S.C., J.M.G., S.V.; resources, J.M.G.; data curation, M.G.G., S.I., J.A.R., F.C.G., J.P., S.C., J.M.G., S.V.; writing—original draft preparation, M.G.G., V.S.G., N.M.R., S.I., J.A.R., F.C.G., J.P., S.C., J.M.G., S.V., X.X.; visualization, X.X.; supervision, X.X.; project administration, F.C.G. and J.M.G.; funding acquisition, J.M.G. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** We have performed all experiments with human subjects based on the use and ethics of the Hospital Universitario de Burgos policy for trials with humans. The ethics committee of clinical experimentation of the region of Burgos, Spain, approved this study (minute 13/2017, internal code: 2017.200) on November 16, 2017. Informed consent was obtained from all participants of the study according to Spanish Organic Law 15/199, December 13, related to Spanish Personal Data Protection Regulation and Royal Decree-Law 1720/2007, December 21.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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