Maleimide-functionalized biopolymers as game changer in wound management

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Abstract

Wounds affect 2-4% of European health care costs and expenditures increase rapidly with complications caused by wound infections. Therefore, proper wound management is required for successful healing of wounded skin. The aim was to synthesize and investigate a modification of alginate for enhanced adhesiveness and antibacterial activity for wound management. Alginate was modified with 1-(2-aminoethyl) maleimide through amide linkage and spectroscopically characterized. Maleimide modified alginate was investigated regarding incorporated maleimide content, in vitro toxicity in terms of erythrocyte viability, as well as adhesiveness to skin and mucosa, influence on skin hydration and moreover antibacterial activity against Escherichia coli. Results of investigation revealed maleimide content of 37.65%. Erythrocyte viability of more than 80% indicated non-toxicity of the modified polymer. Bioadhesion testing exhibited over 8.4 times greater maximum detachment force and 30.2 times elevated total work of adhesion in comparison to the unmodified polymer. Antibacterial activity against Escherichia coli was introduced to the polymer by the modification, as agar disc diffusion assay revealed inhibition zone diameter of 18.5 mm. By these findings, maleimide modified alginate is a promising candidate for development of innovative bioactive polymers in wound management, with advantageous properties such as biocompatibility, advanced adhesiveness and antibacterial activity.

Introduction

In Europe, acute or chronic wounds affect an estimated 1.5-2 million people, whereby 2-4 % of total healthcare expenditure is spend on wound care [1]. The skin plays a crucial role in the continuous maintenance of life. Classification of skin injuries is mainly divided into acute and chronic wounds, both of which require proper care such as adequate wound dressings. Occurrence of complications such as infections with e.g. Escherichia coli lead not only to increasing costs but also to antibiotic resistances and infection-attributable deaths. Wound dressings are available in various forms and are intended to provide an environment that supports the skin's ability of self-healing. Over time, the choice of wound dressing materials has shifted from traditional ones (e.g. gauze) to more promising materials like hydrogels, hydrocolloids, foams, etc. for enhanced wound healing. These are often prepared from biopolymers such as alginate, which can be produced by bacteria and brown algae [2].

Procedures

Agar disc diffusion assay with Escherichia coli

In this disc diffusion method assay discs were exchanged with 5 mg inserts with a 2.5 mm diameter of AL, MA, AL-MA and kanamycin (KA) as positive control.

To create the microbial inoculum of (E. coli) three different colonies from a MHA petri dish containing an E. coli culture were touched with a disposable sterile inoculating loop, transferred into a 15 mL falcon tube and suspended in 3 mL MHB. The incubation parameters of microbial inoculum were set to 24 h and 37°C (Orbital Shaker-Incubator ES-80 Grant-bio, Grant-Instruments Ltd, Cambridge, United Kingdom). In order to create a microbial suspension comparable to 0.5 McFarland 100 µL of the inoculum and 100 µL of 0.5 McFarland were pipetted into a 96-well plate and absorbance was measured at 620 nm. To adjust the microbial inoculum to 0.5 McFarland equally to 1.5 x 108 colony forming units (CFU)/mL the inoculum was diluted with MHB [21]. Afterwards the microbial inoculum was spread-plated on the prepared MHA petri dishes (Greiner Bio-One International GmbH, Kremsmuenster, Austria) using a sterile L-shaped bacterial cell spreader (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). Agar plates were treated with 5 mg inserts of AL, MA, AL-MA and KA in duplicates, followed by incubation at 37 °C for 24 h (Memmert incubator Typ X30-K, Schwabach, Germany). After incubation the diameters of inhibition zones were measured.

Results

In the course of disc diffusion method, in which inserts served as discs, microbial inoculum containing E. coli comparable to 0.5 McFarland was spread-plated on previously prepared MHA plates. The 5 mg inserts of AL, AL-MA, were laid on the surface of the agar plates and set to incubate for 24 h. After the incubation, emerged inhibition zones on the plates with 5 mg inserts of AL-MA, MA and KA were visible, whereas AL showed no inhibition of bacterial growth, as evidenced in Figure 1.

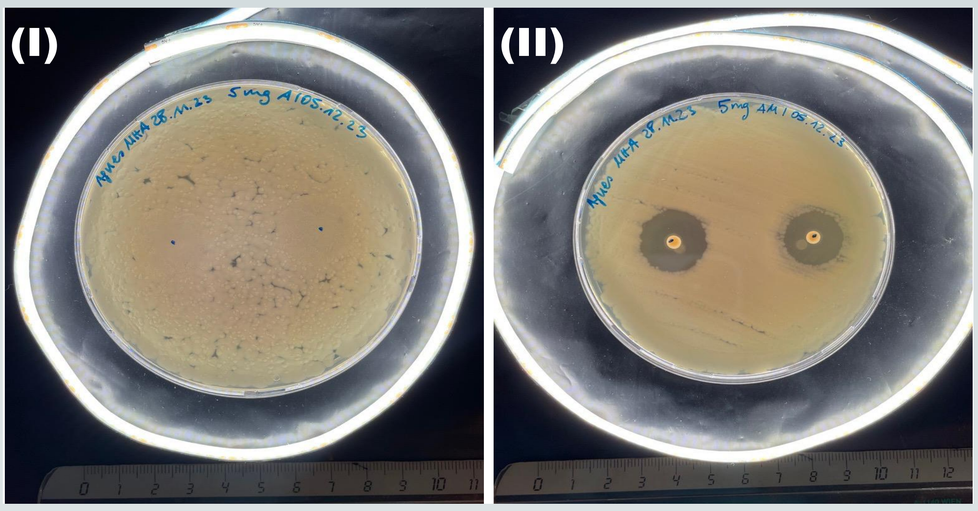

Figure 1: Agar disc diffusion assay with 5 mg inserts of alginate (I) and maleimide modified alginate (II). Inhibition zones formed by maleimide modified alginate (II) were clearly visible, while no inhibition zones were detected for alginate (I).

Inhibition zones were measured, and diameters were evaluated, as indicated in Figure 8. 5 mg inserts of AL formed no inhibition zones, whereby AL-MA, MA and KA exhibited antibacterial activity against E. coli through developing inhibition zones with diameters ranging from 1.85 cm to 4.15 cm, respectively.

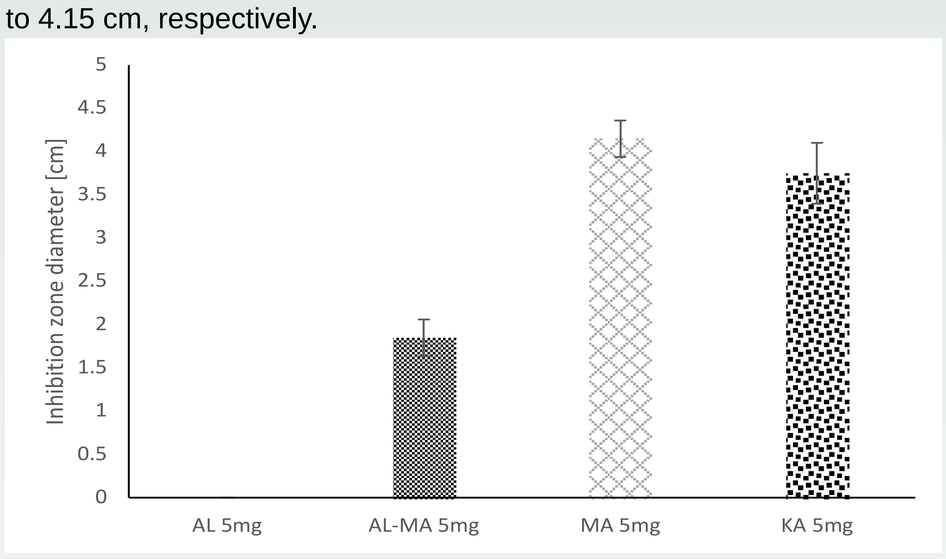

Figure 2: Results of agar disc diffusion assay. Evaluation of measured inhibition zone diameters of 5 mg inserts of alginate (AL), maleimide modified alginate (AL-MA), maleimide (MA) and kanamycin (KA), respectively. Data is shown as means of two measurements (± SD).

Conclusion

Maleimide modified alginate was successfully synthesized and investigated in terms of characterization, degree of modification, biocompatibility, adhesive and hydrating properties, as well as antibacterial activity. Conducted in vitro study showed no toxicity, as erythrocyte viability had an amount of more than 80%. Enhanced mucoadhesion, bioadhesion and skin hydration of AL-MA over unmodified AL were proven by an over 12-fold higher adhesiveness to mucosa, 8.4-fold greater MDF and 30.2-fold elevated TWA on skin. Skin hydration testing revealed normalized trans epidermal water loss values of up to 1.10 g/m2h. In addition, AL-MA exhibited antibacterial activity against E. coli by forming inhibition zone diameter of 18.5 mm in an agar disc diffusion assay. AL-MA as potential wound management candidate might pave the pathway of curing and healing of skin injuries in order to prevent over-usage of oral antibiotics and thereby antibiotic resistances.

References

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