

Article

Translating the Properties of Physicochemical Dressings into Clinical Decision-Making for Heavily Exuding Wounds

Paulina Sánchez-Toledo ^{1,2}, Rosa M. Salgado ¹, Silvestre Ortega-Peña ¹  and Edgar Krötzsch ^{1,*} 

¹ Laboratory of Connective Tissue, Centro Nacional de Investigación y Atención de Quemados, Instituto Nacional de Rehabilitación “Luis Guillermo Ibarra Ibarra”, Calzada México-Xochimilco 289, Colonia Arenal de Guadalupe, Tlalpan, Mexico City 14389, Mexico; psanchez@lasallistas.org.mx (P.S.-T.); salgado_rm@yahoo.com.mx (R.M.S.); silvestreortega@yahoo.com.mx (S.O.-P.)

² Faculty of Chemical Sciences, Universidad La Salle-México, Benjamín Franklin 45, Colonia Condesa, Cuauhtémoc, Mexico City 06140, Mexico

* Correspondence: kroted@yahoo.com.mx; Tel.: +52-155-2106-6140

Abstract

Introduction: Heavily exuding wounds can macerate perilesional skin and favour infection. Information on the properties and use time of dressings should be clear and available to healthcare providers. **Methods:** Our aim was to evaluate the updated series of the AQUACEL[®] dressing family, because their evolution from a single layer of carboxymethylcellulose (NaCMC) to a multicomponent antiseptic or its combination with polyurethane covered with a silicon layer has developed the technology beyond simple exudate absorption. Using gravimetric analysis, we evaluated the porosity, water uptake, and water vapour transmission rate (WVTR) of AQUACEL[®] Ag+Extra, AQUACEL[®] Foam, and Foam Pro. By modifying the method of measuring WVTR, we assessed this outcome during the progressive saturation of the dressings. We also performed a disc diffusion assay on agar to determine the antimicrobial effects of the dressings. **Results:** Independently of porosity, a second layer of cellulose in AQUACEL[®] Ag+Extra doubles water uptake and quadruples WVTR compared to foam forms. When the different dressings were evaluated for WVTR under progressive saturation, we did not observe any statistically significant changes, indicating that retained liquids do not alter dressing properties, which is a more biologically suitable approach. Despite the acidic character of the cellulose hydrofibre contained in the three dressings, the lack of any antiseptics in the foam forms makes them unsuitable for use in colonised or infected wounds, although they can act as a physical barrier for microorganisms and mechanical damage. The opposite results were observed for the AQUACEL[®] Ag+Extra dressing, which contains silver, EDTA, and benzethonium chloride. **Discussion:** Data on the physicochemical composition of dressings can enable healthcare providers to choose the appropriate dressing series to use during wound bed preparation and beyond.

Keywords: wound care; carboxymethylcellulose; ethylenediaminetetraacetic acid; benzethonium chloride; polyurethane; porosity; water uptake; water vapour transmission rate



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1. Introduction

Wound dressings are medical devices designed to cover and protect the wound bed immediately after therapeutic cleansing and debridement [1]. Advanced wound dressings are those that protect the wound bed from contamination with microbes while

retaining exudate secretion, thereby maintaining a moist environment that allows for proper wound closure. In general, these dressings include hydrocolloids prepared with different materials, where the fibres of cellulose and alginate are the most common and budget-friendly materials used in their manufacture. In addition, polyurethanes or polyvinyl alcohol can be used in a simple form or in combination with hydrocolloids with the aim of providing moisture, maximising exudate retention, or avoiding returning exudate to the wound [2]. In particular, regenerated carboxymethylcellulose (NaCMC) has been extensively explored for wound exudate absorption [3], as well as for use as a water carrier when managing dry wounds [4]. NaCMC allows for gelation of the dressing when it comes into contact with the exudate [5], promoting dressing adhesion to the irregular surface of the wound while vertically retaining the excess fluid [6]. Physicochemically, NaCMC exhibits a remarkable water uptake ability while maintaining its physical integrity and forming an insoluble gel structure [5]. To accommodate support for wounds from diverse aetiologies, commercial dressings are continuously being redesigned and optimised. Consequently, understanding how these structural modifications impact the wound microclimate is critical, as clinicians often face challenges when selecting the optimal dressing from a single technological platform for specific wound types. Rigorous physicochemical characterisation is therefore essential to determine their specific clinical indications. Key evaluations include (1) pore size and hydrophilicity, which dictate fluid dynamics and structural affinity; (2) water uptake or swelling ratio, which provides a direct measurement of the device's fluid absorption capacity [7]; and (3) the water vapour transmission rate (WVTR), which indicates the dressing's ability to regulate water evaporation from the exudate [8]. Dressings containing antimicrobials, which use hydration-driven release kinetics [9] or other mechanisms such as adsorption [10], to ensure short- or long-term efficacy against microbial growth are important as infection control remains a critical factor in wound repair. Our group has evaluated different devices and wound management strategies [10–16], and in this work, we evaluate the most recent update of the AQUACEL[®] dressing family series, in order to demonstrate how the different materials can handle wound moisture under clean or infected conditions, focusing on an *in vitro* study of the microenvironment. The first iteration of AQUACEL[®] dressings was a single layer of regenerated NaCMC, modified later with silver embedded into cellulose fibres to retain exudate and treat local infections [6]. Later, it evolved into a multifunctional dressing AQUACEL[®] Ag+Extra, improving exudate retention with a second layer of regenerated NaCMC [17], while strengthening infection and biofilm control through the incorporation of ethylenediaminetetraacetic acid (EDTA) and benzethonium chloride [18]. Subsequently, AQUACEL[®] Foam emerged to protect clean injuries with a high exudate rate in high-pressure zones in resting patients or in exposed regions. This update added a semipermeable waterproof silicone top layer covering an intermediate polyurethane pad over a single layer of NaCMC without antiseptics that gels upon contact with exudate. To keep the device fixed to the treated area, the next update of the dressing included a soft silicon adhesive layer to attach it to the perilesional skin. Finally, with the aim of avoiding the attachment of the bottom of the cellulose to the injury, the final development of this technology, AQUACEL[®] Foam Pro, includes an additional perforated silicone layer in the bottom of the dressing [19]. This characteristic is quite important because cellulose fibres often attach tightly to the wounds, and when clinicians remove the dressing, the newly formed epithelium detaches and reopens the wound [16].

Despite the analyses performed on dressings, no single test can simulate clinical scenarios; furthermore, no comparative evaluation has been carried out regarding the new features being introduced. To bridge this gap and offer healthcare providers and the industry practical insights into the management and scheduling of exudative and infected wounds, we aimed to evaluate *in vitro* the physicochemical properties of three system-

atically related commercial dressings from the AQUACEL[®] series, including a modified testing methodology we designed that simulates the expected microclimate of treated skin, thereby establishing more accurate evidence-based clinical selection criteria. Our results can be used indirectly to intuit the role of other marketed wound dressings prepared with carboxymethylcellulose hydrocolloids, polyurethanes, silicone layers, and antiseptics.

2. Materials and Methods

In this work, we evaluate the updated series of the AQUACEL[®] dressing family, where regenerated NaCMC is the basis of the evolution, which has focused on two approaches. The first approach involves improving the antiseptic properties using antibiofilm strategies (silver, EDTA, and benzethonium chloride) in a double layer of NaCMC, and the second one is related to the management of clean or cleansed chronic wounds that need skin protection, provided by polyurethane foam covered with a silicon layer on a basic single layer of NaCMC; in both cases, cellulose is the main material in the wound dressing. Materials involved in these two design strategies are commonly used by various researchers and/or industries, so this assessment of AQUACEL[®] Ag+Extra, AQUA-CEL[®] Foam, and AQUACEL[®] Foam Pro can inform the medical and industry fields on the roles and importance of these materials, enabling better designs in the future.

2.1. Morphology of Dressings Under Low-Magnification Microscopy

In order to identify the different materials that confer physical properties to the newest members of the AQUACEL[®] dressing family, 1 cm² sections of dry, foetal bovine serum (Gibco; Life Technologies, New York, NY, USA)-hydrated, or haematoxylin and eosin (H&E; Sigma Aldrich, St. Louis, MO, USA)-stained pieces of AQUACEL[®] Ag+Extra, AQUACEL[®] Foam, and AQUACEL[®] Foam Pro (Boston Medical Device de México S. de R.L. de C.V., Mexico City, Mexico) were observed and photographed using a stereoscopic microscope (SteREO Discovery.V20; Carl Zeiss, Göttingen, Germany) fitted with a colour high-speed camera (AxioCam; Carl Zeiss). Photomicrographs were acquired from lateral, upper, and bottom views.

2.2. Gravimetric Analyses to Determine Porosity, Water Uptake Ability, and Water Vapour Transmission Rate of Dressings

For porosity analysis, dressings were cut with a 1.5 cm diameter punch device (ART-SEWPLY, Beijing, China). Diameters and heights were measured using a Vernier digital calliper (INSIZE, Mexico City, Mexico), and dry dressings were weighed on a GX-800 balance (A&D Company. Ltd., Tokyo, Japan) and placed in a 24-multiwell plate with a cap (Corning, New York, NY, USA). Dressings were saturated by immersion in absolute ethanol, and the plate was placed in a vacuum chamber (Desiccator Nalgene[™], Los Angeles, CA, USA; Vacuum pump Feligno, S.A. de C.V., Jalisco, Mexico) at 10 inHg, at room temperature (RT), with 52% relative humidity (RH) (Digital thermo-hygrometer Steren S.A. de C.V., Mexico City, Mexico) for 5 min. Then, excess ethanol was removed via capillary action by gently bringing a paper towel close to the dressings. The dressings were immediately weighed again and porosity was calculated according to

$$\%Porosity = \frac{(Wf - Wi) \times 100}{\rho * V}$$

where Wf and Wi are the final and initial weights of the dressing, ρ is the density of ethanol (0.789 g/mL), and V is the volume of the dressing fragment [7].

Water uptake ability was assessed using dressing samples prepared as before. After weighing each dry sample, dressings were immersed in 2 mL of deionised water for

24 h at RT. Then, the excess water was removed with a paper towel, the dressings were immediately weighed again, and the water uptake ability was calculated according to

$$\% \text{Water uptake ability} = \frac{(W_{\text{wet}} - W_{\text{dry}}) \times 100}{W_{\text{dry}}}$$

where W is the weight of the dressing when dry or wet [7].

For WVTR, we followed the water method detailed in the Standard Test Methods for Water Vapor Transmission of Materials E96 [8]. First, 3000 mm² generic wide-mouthed glass jars were filled with distilled water up to 5 mm from the top; this air space was chosen because it remained possible to handle the jars without direct contact with the water, making the test closer to practical conditions. Then, the jars' mouths were tightly coated with the dry dressings placed face up (as expected during their clinical use), avoiding contact between the water and the material, and leaving only the necessary excess of material for attachment with a rubber band, where the sample and jar were sealed with Teflon tape across the edge. Devices were weighed and incubated at 37 °C in an incubator (Ecoshel 9052, Mexico City, Mexico) under 20% RH for 24 h. Then, the devices were immediately weighed and water loss in the form of vapour was assessed by the difference between the initial and final weights according to

$$\text{WVTR} = \frac{W_i - W_f}{A \times t}$$

where W_i and W_f are the initial and final weights of the dressings, A is the area of the jar's mouth, and t is the time of the test; values obtained are expressed as g/m²·24 h. As a blank test, a dummy specimen consisting of a jar filled with water and hermetically closed with its cap was weighed at the beginning and 24 h later; no significant changes were observed between weights.

In order to know whether water vapour transmission changes while the dressing absorbs exudate, an in vitro approach to the clinical performance of wound dressings was designed as a modification of the analysis of WVTR, where the assessment was performed during progressive dressing saturation. The device used for the assay is shown in Figure 1. The device is essentially the same as that used for WVTR, but in this case, a needle was inserted through the dressing, through which 5 mL of the liquid was added every hour for 5 h. Additionally, a drilled Petri dish, with the bottom and lid glued on their external sides, was placed over the dressing. Finally, a metal mesh covered with a paper towel was positioned inside the modified Petri dish, where 3 g of CaCl₂ was left for water vapour capture. The mesh with the paper towel and CaCl₂ was weighed at the beginning of the experiment and every hour until the fifth hour. WVTR under progressive saturation was determined as above, except for the duration of the experiment, which was a 1 h instead of a 24 h period (g/m²·h). To determine the effect of plasma components (exudate) on WVTR under progressive dressing saturation, we performed two experiments: the first with water and the second with diluted plasma (1:1 v/v). Experimental control was performed using AQUACEL® Ag+Extra without saturation, where WVTR determinations were recorded at 1, 2, 3, 4, and 5 h. As an internal control, a Petri dish with CaCl₂ was weighed at the beginning of the experiment and every hour; no significant changes were observed among weights. Cross-sections of the centre of every dressing were observed hourly and photographed as before.

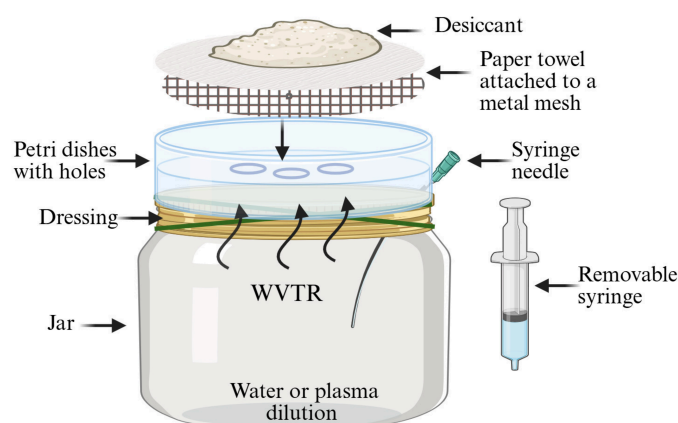


Figure 1. System designed to evaluate water vapour transmission rate during progressive saturation. The different dressings were progressively hydrated with water or plasma every hour over a period of time. Created with BioRender.com.

2.3. Antimicrobial Assay of Dressings

To evaluate the anti-microbial activity of the dressings, clinically isolated and typified *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* strains were used in a disc diffusion assay on agar [20]. Microorganisms isolated from infected wounds belong to the microbial collection of the Laboratory of Connective Tissue; Instituto Nacional de Rehabilitación “Luis Guillermo Ibarra Ibarra”, Mexico City, Mexico. First, 1.5×10^8 UFC/mL of *S. aureus*, *Ps. aeruginosa*, and *C. albicans* were inoculated onto tryptic soy agar plates. Afterward, 5 mm diameter cylindrical sections of the dressings or controls were carefully placed on the plates and incubated under aerobiosis at 37 °C for 24 h. After incubation, inhibition growth zones were measured. Positive and negative controls were sterile filter paper discs with and without iodopovidone solution (iodopovidone solution 0.1 g/mL; Laboratorios Jaloma SA de CV, Jalisco, Mexico). Using the images acquired at $4.7\times$ with a stereoscopic microscope (Carl Zeiss) fitted with a colour high-speed camera (Carl Zeiss), the growth inhibition zones were outlined, and areas were assessed using ImageJ 1.54P software [21].

2.4. Statistical Analysis

Data represent triplicates from two independent experiments ($n = 6$) for porosity, water uptake, WVTR, and anti-microbial activity. For WVTR during progressive dressing saturation, duplicates from two independent experiments ($n = 4$) were analysed. One-way ANOVA was chosen for gravimetric assays because the study has only one categorical independent variable (type of dressing) with three groups. To demonstrate differences among the groups, we performed Tukey’s post hoc multiple comparison test. Additionally, the criterion was strengthened when the paired data exhibited normal behaviour and were corrected using the Geisser–Greenhouse method. For antimicrobial activity, since the positive control, AQUACEL® Foam, and Foam Pro had no effect, statistical comparison was performed only between the positive control and AQUACEL® Ag+Extra using a paired t test. Statistical analyses and data plots were performed with GraphPad Prism 8 (GraphPad Software, Boston, MA, USA).

3. Results

3.1. Cellulose Hydrofibre, Polyurethane Foam, and Silicone Sheets Confer the Structural Properties of the Dressings, Along with Their Acidic Character and Exudate Retention

Although the composition of the different dressings evaluated in this work has been extensively described by others [22,23], here we show photomicrographs from different

views of the dressings at low magnification. The AQUACEL[®] Ag+Extra photomicrograph revealed the stitched cellulose fibres, different from the single layer of cellulose fibres adhered to the polyurethane foams in AQUACEL[®] Foam and Foam Pro; only in AQUACEL[®] Foam Pro are cellulose fibres contained by a perforated sheet of silicone with holes of approximately 0.75 mm, separated from each other by less than 2 mm. In addition, AQUACEL[®] Foam and Foam Pro are covered with a single silicone sheet (Figure 2, upper panels, “Dry”). When dressings were embedded with serum, it was mostly retained in the cellulose fibre layer, with slight diffusion to the polyurethane foam (Figure 2, middle panels, “Serum”). Also, the acidic and basic/neutral character of the cellulose hydrofibre or polyurethane foam (purple and pink, respectively, in the lower panels of Figure 2) was demonstrated using H&E staining.

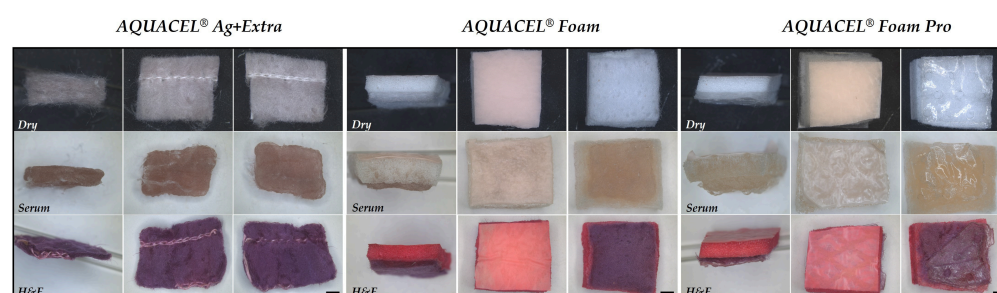


Figure 2. Morphology of the different dressings when dry, serum-impregnated, and H&E-stained. Vertical panels show lateral views, upper views, and bottom views from left to right, respectively. Bars represent 2 mm.

3.2. Structure and Composition Dictate the Main Physical Properties of Dressings

The porosity test method used here was based on the assay reported by others in scientific papers [7] also assessing materials used for wound dressings. The higher porosity observed in the dressings containing polyurethane, when compared with cellulose fibres alone, was associated with the foam structure. AQUACEL[®] Ag+Extra contains a double layer of cellulose fibres, and its closed arrangement generates 50% of the porosity, while AQUACEL[®] Foam and Foam Pro contain a single layer of cellulose fibres, in addition to the polyurethane. This structural combination means that the foam content increases the porosity up to 75% (Figure 3). Despite the higher porosity of the foam dressings, the double-cellulose-fibre dressing showed twice the water uptake ability compared to the other dressings (Figure 3). Despite the negligible difference between both foam dressings, there is a slight but statistically significant increase and decrease in porosity and WVTR (Figure 3), respectively.

3.3. Progressive Dressing Saturation with Water or Plasma Does Not Affect the Water Vapour Transmission Rate

WVTR is a standardised method based on the ability of a dry cover to allow for vapour transmission [8]. However, when a dressing is placed on an exudative wound, the dressing becomes saturated; therefore, we wondered whether pore occlusion could affect vapour transmission. To demonstrate such possible changes, we developed a device to measure WVTR by progressively saturating the dressings that covered the jars (Figure 1). First, we assessed the dry dressing and observed a significant difference in WVTR compared to the method previously used, due to the measurement of the weight difference between the beginning and the end of the evaluation time, because the water vapour was captured by the desiccant instead of being released into the environment. Once we understood the system's behaviour, we evaluated WVTR during progressive dressing saturation with water (Figure 4a) or with diluted plasma (Figure 4b), and we observed no difference among

the samples. In fact, there was no statistically significant change between water or diluted plasma saturation at any time tested, even though the dressings swelled progressively (Figure 4c).

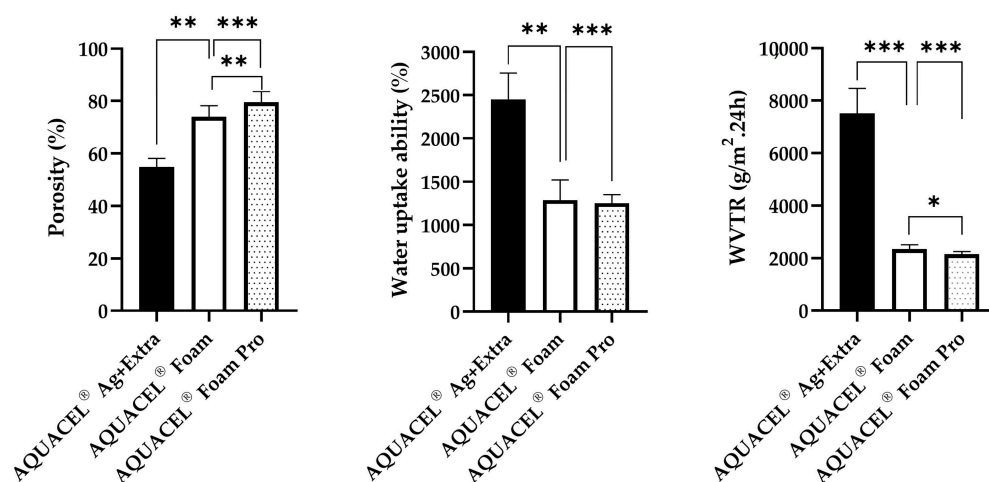


Figure 3. Porosity, water uptake ability, and water vapour transmission rate (WVTR) of the different dressings were determined using gravimetric methods. Statistically significant differences were observed between dressings according to one-way ANOVA and Tukey's multiple comparison test; * p value < 0.02, ** p < 0.007 and *** p value < 0.0004.

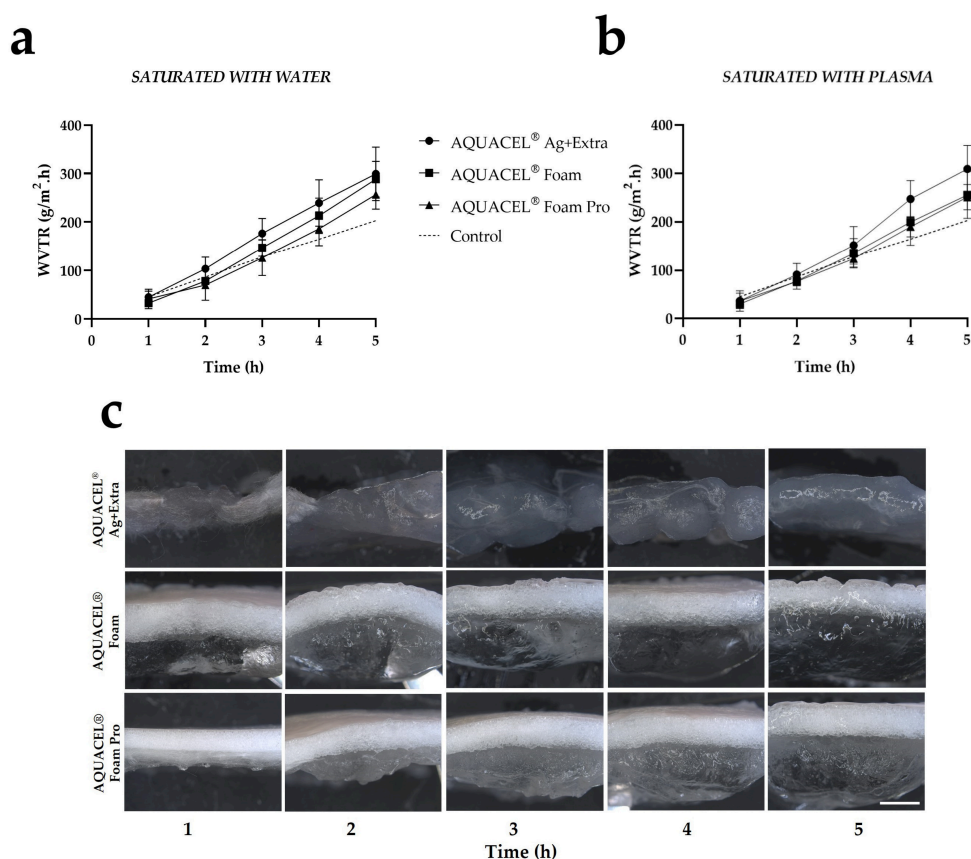


Figure 4. Water vapour transmission rate (WVTR) of the different dressings assessed during progressive saturation. Charts show the kinetics of WVTR of the different dressings during progressive saturation with (a) water or (b) diluted plasma (1:1 v/v). No statistically significant changes were observed among groups at the same time point. (c) Morphology of dressings under water saturation during 5 h; bar represents 2 mm.

3.4. Hydrofibre Antiseptic Release Prevents Microorganism Growth

Since only AQUACEL® Ag+Extra contains cellulose fibres embedded with an antiseptic/antibiofilm mixture, the disc diffusion assay revealed the inhibition of microbial growth (Figure 5) beyond the dressing border (Figure 6), even around the dishevelled fibres (Figure S1). The cellulose fibre dressing was more effective against *C. albicans* and *Ps. aeruginosa*, and the effect was reduced when cultured with *S. aureus*. Interestingly, every AQUACEL® Ag+Extra fibre looks like it is sufficiently embedded with antiseptic, since the diffusion assay showed individual effects when fibres spread out from the dressing disc (Figure S1). Conversely, AQUACEL® Foam and Foam Pro were limited in their ability to control microbial growth, even with the acidic character observed in the cellulose component, as previously described here (Figure 2, lower panel, H&E).

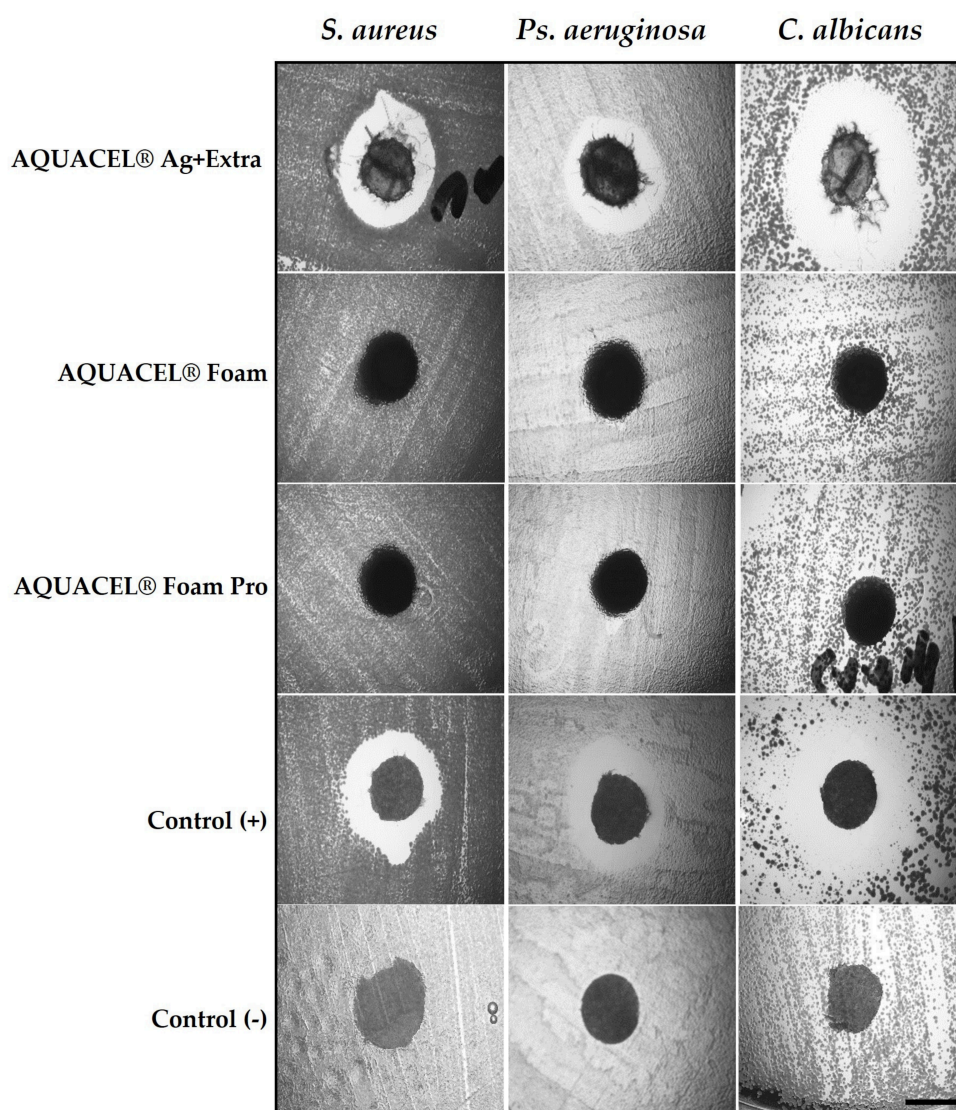


Figure 5. Photomicrographs from the disc diffusion assay. Agar cultures of *S. aureus*, *Ps. aeruginosa*, and *C. albicans* were incubated with the different dressings for 24 h. Inhibition zones are shown by the lack of microorganism growth (translucent areas). Photomicrographs were obtained from the bottom of the dishes. Positive (+) and negative (−) controls were paper discs with and without iodopovidone solution; the bar represents 5 mm.

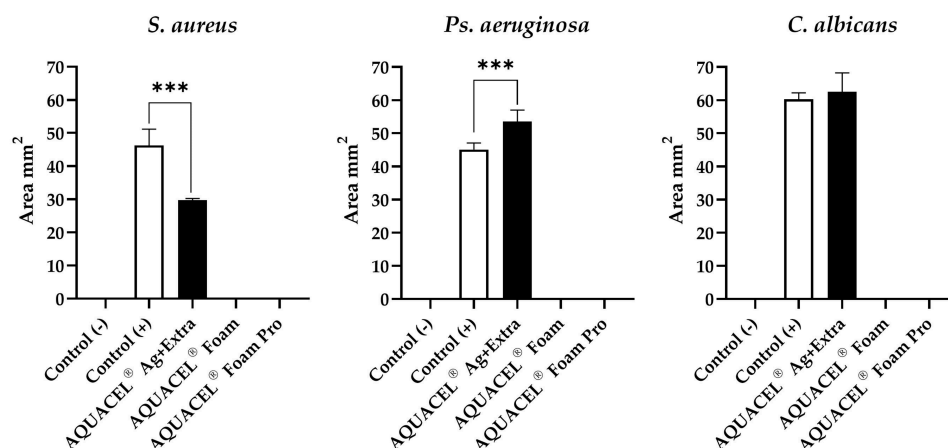


Figure 6. Inhibition of microbial growth with the different dressings was determined using the disc diffusion assay. Charts show growth inhibition zones of the different dressings when incubated in agar cultures of *S. aureus*, *Ps. aeruginosa* and *C. albicans*. Statistically significant differences were observed between dressings according to one-way ANOVA and Tukey's multiple comparison test; *** p value < 0.0004. Data were obtained only from cultures with homogeneous growth inhibition zones ($n = 6$).

4. Discussion

Rebuilding the damaged skin barrier is a multifactorial process involving, at the outset, moisture and inflammation controls. An excess of exudate causes the maceration of perilesional skin, while dry wounds lack the vehicle for cell stimulation and become rigid, creating scabs; both conditions impair wound healing [24]. Physiochemically, NaCMC exhibits a remarkable water uptake ability [5] while forming an insoluble gel structure [5], allowing high water vapour levels to escape, which is useful for maintaining an appropriate moisture environment for highly exudative wounds. Nevertheless, mechanical protection of the wound is not sufficient in patients wearing NaCMC dressings (AQUACEL® Ag+Extra) alone, especially when injuries are in high-pressure zones in resting patients or in exposed regions, such as the malleolus, among others. For that reason, polyurethane foams, which have the double porosity of the cellulose fibres as presented here, have been used for mechanical skin protection, in addition to their wound fluid retention ability [25]. In this manner, the combination of NaCMC and foams can improve the handling of patients with clean injuries and wound fluid management simultaneously. Here, we observed that water uptake and WVTR were statistically significantly different when comparing the combined dressings (AQUACEL® Foam and AQUACEL® Foam Pro) to NaCMC alone. This effect is related to the capacity of NaCMC to retain water by gelation, while polyurethanes only allow water to spread through the material. Regarding the WVTR behaviour of the different dressings, AQUACEL® Ag+Extra exhibited almost a four-fold proportion compared to AQUACEL® Foam and Foam Pro. In this case, thickness is not the key factor; the release of vapour through the materials can be dictated by the nature of the polymer itself, as well as by the upper silicon layer contained in the Foam forms of the dressings. It is clear that despite the polyurethane's water uptake ability, it cannot match the effect of the second layer of NaCMC in the AQUACEL® Ag+Extra dressing, even when it is possible to visualise the infiltration of serum in the polyurethane layer of AQUACEL® Foam and Foam Pro. This effect reflects the wide difference regarding exudate absorption among synthetic polymers, such as polyurethane or polyvinyl alcohol, vs. hydrocolloid formation.

Since exudate is the secretion of body fluids towards the wound coming from intra-cellular and extracellular tissue components, such as water, electrolytes, cellular debris, and proteins [26], it is to be expected that progressive dressing saturation could impair

water vapour permeability; consequently, when a dressing remains on a wound for long periods, it could macerate the surrounding skin. During the wound dressing evaluation, we observed that the general assays for exudate management required a link between the water lock of the dressing and the water vapour release, with progressive dressing saturation being a real condition. Thus, we modified the WVTR assay to evaluate the progressive saturation. Nevertheless, when the three dressings assessed in this work were evaluated for their WVTR under progressive liquid saturation, it was demonstrated that all of them allowed water vapour to escape continuously in a similar fashion when saturated with either water or an exudate equivalent (diluted human plasma). This result is relevant since the structural and physical properties of the dressings are appropriate for highly exudative wounds, such as venous ulcers, among others [24]. Although the method designed here for WVTR during progressive dressing saturation is based on the same rationale as the classical water or desiccant methods, it was evident that the values obtained did not match those of the initial method used. However, by including AQUACEL® Ag+Extra without saturation as an experimental control, the data showed that during the 5-h period, dressings becoming embedded with water or diluted plasma did not affect their ability to evaporate water.

Despite gravimetric analysis for porosity, water uptake, and WVTR being the gold standard to assess those characteristics of dressings for wound management, the various methods of assessment make it difficult to choose the appropriate dressing [27]. The final goal for exudative wound management should be the practical use of a dressing for which clinicians are aware of exudate absorbency, wear time, and prevention of maceration [28]. Nevertheless, the different methods of expressing the analytical results and the devices or laboratory apparatuses used for this purpose become impractical for comparing all of the available products; however, it is possible to intuit their differences. For example, in this work, we express the percentage of water uptake, informing clinicians that AQUACEL® Ag+Extra can retain almost 25 times its weight in water, while allowing approximately 8 kg of water to evaporate from every m² of dressing in 24 h; therefore, if the dressing is 10 × 10 cm (100 cm²), the water vapour loss would be 80 g in 24 h, similar to the results observed by others [29]. In summary, it is obvious that most exudative wounds require the most absorbent dressings; however, the knowledge of every exudate rate observed in chronic wounds dictates which dressing can be used and for how long, based on the liquid retention and water evaporation of the dressings, resulting in low perilesional skin maceration and moisture balance. Besides the physical role of chronic wound exudate, biochemically, it is deleterious to wound improvement when excessive, because it contains high proportions of inflammatory mediators, such as cytokines that reduce fibroblast mobility [30], along with protease overexpression that prevents granulation tissue formation and epithelialization [31], which can be mitigated with the exudate absorption effect using the appropriate dressings.

Moreover, chronic wound management also requires microbial control, whether because the injury is infected or because the constant high exudate predisposes it to contamination. Infection is the most common cause of non-healing in acute and chronic wounds [32]. The excessive presence of microorganisms (microbial bioburden) within the wound lengthens the inflammatory phase and delays the healing process [33], as demonstrated by the correlation between a wound's microbial bioburden and its healing trajectory [34]. To promote the healing of infected wounds, clinical guidelines recommend taking wound hygiene measures to reduce microbial load [32]. Wound hygiene is achieved by sequential steps including cleansing, debriding, reshaping the wound edges, and dressing the wound [35,36]. Antimicrobial dressings are indicated to cover wounds with local infection; they serve as a physical barrier against the invasion of new microorganisms and inhibit the growth of residual microbial bioburden [34,36].

Despite the acidic character of NaCMC [3] observed when stained with haematoxylin and eosin, it does not inhibit microbial growth by itself. In this study, the concentration of silver and EDTA in AQUACEL® Ag+Extra was not evaluated; however, according to the technical datasheet of the dressing, the approximate concentration of ionic silver (Ag^+) is 1.2% *w/w* (1 mg/cm²), while the concentration of EDTA is not reported [37]. The experimental papers show that dressings with silver can reduce the viability and proliferation of keratinocytes and fibroblasts, and induce oxidative stress and DNA damage in *in vitro*, *ex vivo*, and animal models, and the effect depends mainly on Ag^+ release and not only the total metallic silver content [38–40]. However, the clinical extrapolation of these findings remains uncertain, because the biological components of the wound bed, such as exudate or extracellular thiols, can partially neutralise both the cytotoxicity and the antimicrobial activity of the silver [38,41]. Clinical studies on AQUACEL® Ag, the previous version of the dressing assessed here, did not show a delay in wound healing, so it can be proposed that the wound environment modulates the practical high cytotoxicity of silver [42], which supports its recurrent use in different treatments.

The NaCMC used for AQUACEL® Ag+Extra slowly released the silver ions from the dressing to the exudate for up to 2 weeks while entrapping microorganisms within the fibres [6], another physical effect of hydrocolloids. During the evaluation of AQUACEL® Ag [43], the dressing was classified as fast-acting because it releases substantial amounts of Ag^+ in the first 6 h. The authors also classified it as a sustained-release dressing because concentrations of $0.24 \pm 0.01 \mu\text{g}/\text{cm}^2$ were detected in the culture medium at 24 h.

Furthermore, the addition of EDTA and benzethonium increases the speed of planktonic antimicrobial activity when compared with other silver-containing dressings based on NaCMC [44], although their main focus is the prevention and eradication of biofilms [18]. Here we observed that only AQUACEL® Ag+Extra demonstrates antimicrobial activity, as the foam-supplemented forms do not contain any silver, EDTA, or benzethonium. Considering that we used iodopovidone as a positive control, the inhibition of growth of *Ps. aeruginosa* and *C. albicans* was remarkable. Interestingly, some of the inhibition zones were irregular when treated with AQUACEL® Ag+Extra, and this effect seemed to come from the antiseptics embedded in the dishevelled fibres of the dressing, demonstrating their homogeneous distribution in the fibres. The Foam forms of the AQUACEL® series, conversely, can only form a physical barrier against microorganisms, due to their upper silicon vapour-permeable layer, but they should not be used on colonised or infected wounds. While the *in vitro* biofilm assay was not performed in this study, the clinical management of wound biofilms relies primarily on mechanical debridement for physical disruption [45]. In this context, antimicrobial dressings serve to eradicate residual planktonic bacteria released post-debridement, thereby preventing secondary biofilm formation [35].

5. Conclusions

In this work, we evaluated the latest update of the series of AQUACEL® dressings as a model of interaction of different components involved in moisture balance and antiseptics for wounds. We concluded that initial wound evaluation should involve more than the wound itself, because if clinicians can calculate the weight of exudate when removing the first primary dressing from the patient and pay attention to its appearance and odour, they can obtain valuable information regarding the needs for holistic wound management. With this information and the knowledge of the different dressing properties, based on their physicochemical composition, healthcare providers would have the most relevant data required when choosing the appropriate dressing for wound bed preparation and beyond, instead of using a simple gauze or the inappropriate dressings frequently chosen for their antiseptic properties, which could impair tissue repair when used for long

periods, especially when the injury does not exhibit the signs and symptoms of infection. AQUACEL® Ag+Extra or equivalent dressings are particularly recommended for infected and highly exudative wounds after debridement because they demonstrate microbicidal and antibiofilm properties, while retaining abundant exudate by favouring only the moist environment. Conversely, AQUACEL® Foam and Foam Pro or equivalent dressings are recommended for clean or cleansed exudative wounds located in areas of the body with the highest frequency of impact.

Using the information reported here, R&D scientists, clinicians, and marketing professionals can determine the possible roles and applications of different dressings based on celluloses and polyurethane structures in terms of moisture balance.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/scipharm94030076/s1>, Figure S1. Antiseptic diffusion was evident even from single fibres; bar represents 5 mm.

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Abbreviations

The following abbreviations are used in this manuscript:

WVTR	Water vapour transmission rate
EDTA	Ethylenediaminetetraacetic acid
NaCMC	Sodium carboxymethylcellulose

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