COLLAGEN-BASED BIOACTIVE WOUND DRESSINGS LOADER WITH BASIL ESSENTIAL OIL AND HYDROXYAPATITE

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This study aims to explore the integration of hydroxyapatite and basil essential oil (EO) into a collagen matrix to develop novel bioactive wound dressings. By evaluating the structure of the resulting experimental samples and their surface characteristics, we aimed to determine whether they are suitable for general wound management. Our results show that although surface roughness can affect wettability, the addition of hydroxyapatite and basil essential oil significantly improved this parameter. The results demonstrate the distinctive characteristics of the composite samples, making them viable options for wound management. Thus, the sample with the highest amounts of hydroxyapatite and EO (P3) was highlighted by an interesting HAp distribution, increased hydrophilicity, and roughness leading to the conclusion that it is an interesting proposal as wound dressings that require good absorption. Applications were found that were specifically tailored, and each composite had significant advantages.

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1. Introduction

The increasing incidence of surgical wounds, chronic skin wounds, and acute trauma represents therapeutic challenges regarding wound care [1–4]. The risk of infection, which significantly impedes healing and raises morbidity and death rates, is one of the primary issues in wound care. Antimicrobial dressings are now a practical approach to wound healing and hospital cost reduction. Selecting the right antimicrobial dressing requires considering many variables, including the wound type, location, and status as well as its microbiological load and cost. Prevention of infection is one of the main goals of wound care. As it is presented in Fig. 1 there are several types of wound dressings [5–7].

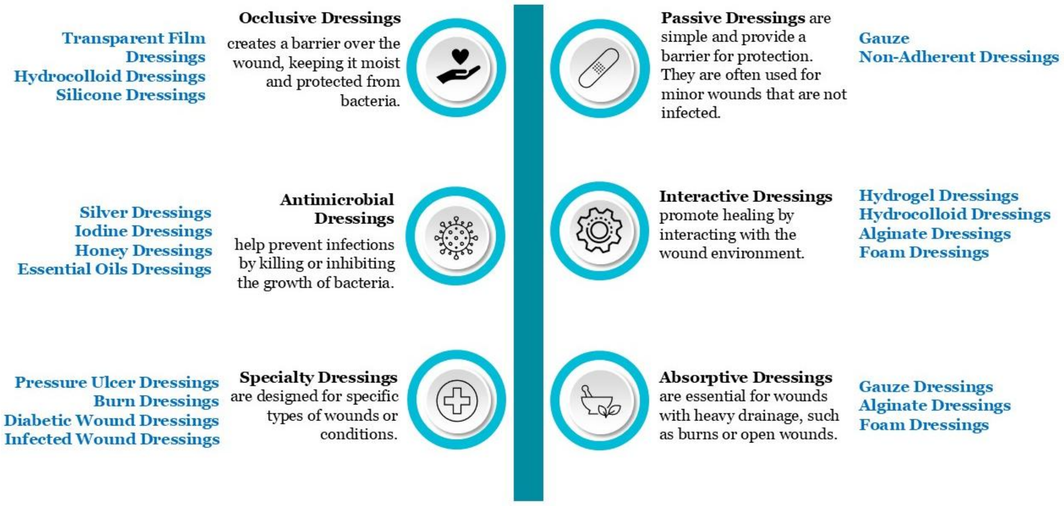

Fig. 1. Wound dressing types

Passive dressings are simple and provide a protection barrier. They are often used for minor wounds that are not infected. In this category are ***gauze*** (a breathable material that allows the wound to aerate but can also absorb excess drainage) and ***non-adherent dressings*** (designed to prevent the bandage from sticking to the wound, which can be painful when removed). There are also **interactive dressings**, that promote healing by interacting with the wound environment. In this category, there are ***Hydrogel dressings***, that are made from hydrophilic polymers such as polyvinyl alcohol, polyethylene glycol, and agar. Hydrogels maintain a moist environment, can absorb a certain amount of exudate, and provide cooling and pain relief. They also contain a gel that helps keep the wound moist, which is essential for healing. They are suitable for dry to minimally exuding wounds [8]. ***Hydrocolloid dressings*** are typically composed of gel-forming agents such as carboxymethyl cellulose, pectin, and gelatine. Hydrocolloids provide a moist healing environment by forming a gel upon contact with wound exudate. They are occlusive, protecting the wound from external contaminants and reducing pain by maintaining a moist environment [9].

Alginate dressings are derived from brown seaweed and are composed of calcium and sodium alginate. They are highly absorbent, forming a gel upon contact with wound exudate. They are effective for moderate to heavily exuding wounds and can assist in maintaining a moist environment. These dressings are prescribed on both infected and non-infected wounds because they can absorb up to 20 times their weight in fluid. Alginate dressings should not be used in dry or slightly drained wounds due to their high absorption capacity [10,11]. **Foam dressings** are designed to absorb drains and protect the wound from external trauma. They are often used for pressure ulcers or burns. They are also utilized, usually in wounds with moderate-to-heavy exudate, to provide a moist wound environment. Because of their versatility and ability to cushion, these dressings are appropriate for locations with higher friction or bony prominences. **Absorptive dressings** are essential for wounds with difficult drainage, such as burns or open wounds. In this category, we can count ***alginate dressings***, ***foam dressing,*** and ***gauze dressing***. **Occlusive dressings** create a barrier over the wound, keeping it moist and protected from bacteria. There are ***transparent film dressings (***made from a thin, clear film that allows the wound to be monitored without having to remove the dressing), ***hydrocolloid dressings,*** and ***silicone dressings*** (made from a soft, pliable material that is gentle on the skin and can be left in place for several days).

Antibacterial dressings are designed to prevent or treat infections and promote healing. They incorporate various antimicrobial agents and biomaterials to enhance their effectiveness. Several antibacterial agents have been used in wound dressings. Silver nanoparticles and silver ions are frequently incorporated into dressings due to their broad-spectrum antimicrobial activity [12]. Manuka honey has strong antibacterial properties due to its high sugar content and release of hydrogen peroxide. Chitosan is a biopolymer with intrinsic antibacterial properties, often combined with other agents to enhance effectiveness [12]. Plant-derived oils (e.g., tea tree, eucalyptus, thyme, and lavender oils) have antimicrobial, anti-inflammatory, and wound-healing properties. Zinc oxide, titanium dioxide, and other metal oxides are used for their antimicrobial and anti-inflammatory properties [12]. Extracts from plants like Aloe vera, neem, and curcumin have been integrated into wound dressings for their antimicrobial and healing properties.

For wound dressing, Polyvinyl alcohol (PVA), polyethylene glycol (PEG), and chitosan for hydrogels were used as biomaterials, because they provide a moist environment and can be infused with antimicrobial agents [12]. Carboxymethylcellulose (CMC), gelatine, and pectin have been used in occlusive dressings that absorb exudate and protect the wound [12]. Electrospun nanofibers made from polymers like polycaprolactone (PCL) or poly(lactic-co-glycolic acid) (PLGA) have been used to create highly absorbent and antimicrobial dressings [12]. Calcium alginate and sodium alginate made from seaweed extracts, often combined with silver or other antimicrobial agents were used for alginate dressings. Polyurethane foam provides cushioning and is often combined with antimicrobial agents like silver or iodine to create foam dressings. Collagen from bovine, porcine, or marine sources promotes wound healing and can be infused with antimicrobial agents to create collagen dressings [12]. The excessive use of antibiotics has led to the emergence of multidrug-resistant bacteria, making many antibiotics ineffective. Some antibiotics often target specific bacteria, whereas alternative agents like silver or essential oils have broad-spectrum activity. There is increasing interest in naturally derived agents such as essential oils, which are less likely to promote resistance and offer additional benefits such as anti-inflammatory and wound-healing properties [8,12]. Essential oils disrupt bacterial cell membranes, inhibit enzyme activity, and affect bacterial metabolism. They are effective against a wide range of bacteria. Many essential oils also have anti-inflammatory, antioxidant, and analgesic properties, making them ideal for wound healing. Essential oils like tea tree oil, thyme oil, and eucalyptus oil have demonstrated potent antibacterial effects, making them attractive alternatives to traditional antibiotics in wound care applications.

2. Materials and methods

2.1. Synthesis of the experimental samples

The composite samples are based on a collagen gel (concentration of 1.77% (w/w), pH=1.8) extracted from calfskin, according to the protocols established at the Collagen Research Department from the Division of Leather and Footwear Research Institute [13]. Because essential oils are volatile, they were introduced in different amounts of hydroxyapatite. By modifying the amounts of hydroxyapatite and basil essential oil, the aim was to obtain experimental samples with good absorption properties while maintaining relative flexibility. To enhance the collagen's therapeutic potential, particles of HAp (from Sigma-Aldrich Chemie GmbH, purity of ≥90%) were incorporated along with different amounts of basil essential oil - basil EO - (from S.C. Hofigal Export Import S.A.). The pH of the obtained compositions was adjusted to a value of 7 using 1 M sodium hydroxide, under continuous stirring. Afterwards, the compositions were lyophilized (*Table 1*).

Table 1

The compositions of the designed experimental composites

Sample	Collagen gel [g]	HAp [g]	Basil EO [µl]
P1	28.24	0.005	0
P2	28.24	0.005	5
P3	28.24	0.125	12.5

2.2. Characterization of the experimental samples

Scanning Electron Microscopy (SEM)

A Quattro S Scanning Electron Microscope (Thermo Fisher Scientific, Oregon 97124, USA) was used to evaluate the structure of the polymeric matrix, additives, and essential oil dispersion.

Contact Angle

The experimental KRÜSS DSA30 Drop Shape Analysis System measured the contact angles of the wound dressings. Since the collagen-based matrices were designed to be in contact with biological fluids, distilled water was used as the liquid to determine the contact angles. The contact angle values were provided as averages.

Roughness

The Form Talysurf® i-Series PRO Range from Taylor Hobson is the instrument used to measure surface roughness. The roughness meter uses Metrology 4.0 Software and consists of a transducer with a standard probe for measuring flat surfaces. Analysing a biomaterial's surface roughness can reveal details about how it behaves in the human body. Measures such as Ra (roughness average) and Rt (the separation between the highest and lowest points of the profile along the evaluation length) were used to assess the surface roughness.

3. Results and discussion

3.1. Experimental sample characterization

The lyophilized samples, presented in Fig. 2, had a spongy texture and a yellowish colour.

Fig. 2. Macroscopic images of the samples P1 (a), P2 (b) and P3 (c) sponges

The previously presented investigation methods were performed on experimental samples with dimensions of 10 mm x10 mm x 5 mm. Regarding the experimental wound dressings, the SEM images revealed comprehensive details regarding the porosity, and component distribution (hydroxyapatite, and basil EO) in the collagen matrix, as well as how these may affect the dressing's effectiveness [14,15].

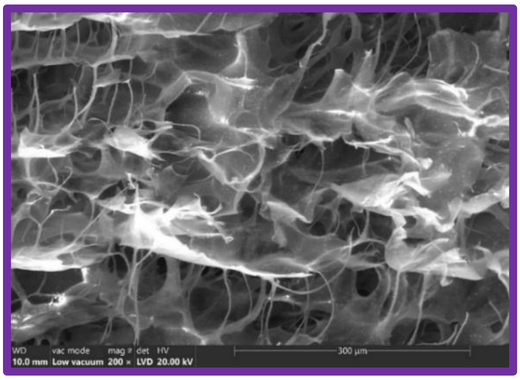

Fig. 3. The structure of P1 sample at 200X magnification

Sample P1 revealed a porous collagen matrix with a limited distribution of HAp particles.

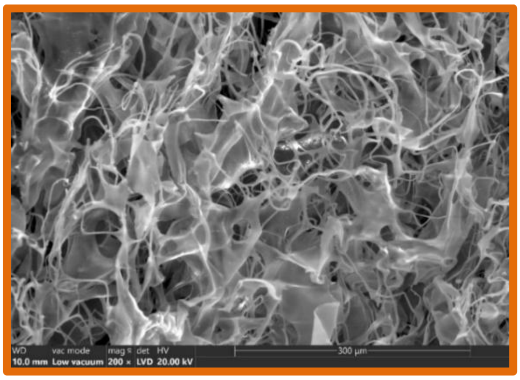

Fig. 4. The structure of the P2 sample at 200X magnification

The collagen structure exhibits a porous and fibrous network, which will aid cell infiltration, but with limited inorganic (HAp) presence. To assist tissue healing, the porosity of the samples is necessary for both fluid absorption and cellular penetration [16]. The distribution of HAp is not very homogeneous, leading to clusters in some areas and bare collagen regions in others.

In Fig. 4, the SEM image shows a slight modification in the sample structure compared to sample P1. The addition of basil EO leads to a decrease in the samples' porosity. However, the low HAp content (same as P1) does not induce a significant change in overall morphology, compared to P1. The presence of small agglomerates of HAp particles in the walls of the collagen network is observed [17].

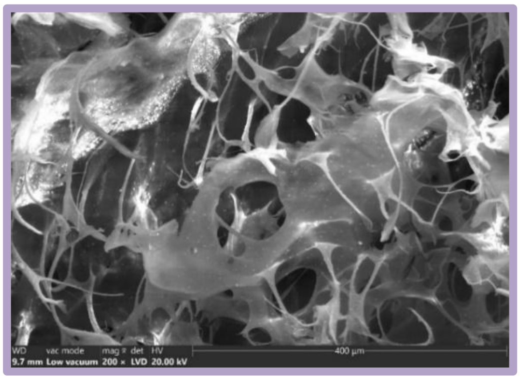

Fig. 5. The structure of sample P3 at 200X magnification

The P3 sample shows a more noticeable distribution of HAp (as seen in Fig. 5), as the amount of HAp is significantly increased. The SEM image reveals a rougher and more heterogeneous structure compared to P1 and P2, with HAp clusters more prevalent. As we can observe in Fig. 5, this sample presents the largest pore size, and we can conclude that this is the influence of the amount of hydroxyapatite. The high HAp content (hydrophilic) combined with a higher amount of basil EO (hydrophobic) creates a more mixed surface [18].

3.2. Surface properties of the experimental samples

Contact Angle

The contact angle values of a material are a key indicator of its wettability, which directly influences how liquids (such as wound exudate or body fluids) interact with the material [19]. In the context of bioactive wound dressings, the contact angle is used to determine whether the material is hydrophilic (absorbs moisture) or hydrophobic (repels moisture), which can affect its ability to promote healing [20].

Table 2

Contact angle values for the experimental samples

	P1	P2	P3
Contact angle [Â°]	90.48	104.22	82.14
Standard deviation	11.66	16.31	7.17

The collagen provides a hydrophilic matrix, while hydroxyapatite is a bioceramic known for its hydrophilic nature which supports cell attachment and promotes wound healing. The contact angle values of sample P1 (90,48°) suggest that the collagen matrix and hydroxyapatite moderately balance the wetting properties. The contact angle value of the P1 sample suggests it could support wound exudate absorption while maintaining a suitable environment for wound healing. The P2 sample exhibits a higher contact angle, indicating an increased hydrophobicity compared to P1. The addition of basil EO, which is hydrophobic due to its content, significantly affects the wettability. While collagen and hydroxyapatite contribute to the hydrophilic properties of the sample, the addition of basil EO induces the formation of a hydrophobic layer on the surface. Basil EO components, like linalool and eugenol, are non-polar, contributing to the increased contact angle value [21,22]. The higher hydrophobicity could reduce the surface's capacity to absorb fluids, which may limit exudate management but can be beneficial in creating a barrier against microbial infiltration and reducing wound infection risk due to the antimicrobial properties of basil EO [23].

The P3 sample has the lowest contact angle value between all samples, indicating an increased hydrophilicity. Although it contains a higher percentage of basil EO, the increased hydroxyapatite content seems to control the surface properties. It is desirable to reach a balance between the amounts of hydroxyapatite and basil EO to obtain wound dressings with hydrophilic properties suitable for clinical needs. The higher hydrophilicity of sample P3 sample suggests improved fluid absorption, which is essential for wound exudate management. The increased hydroxyapatite content can enhance bioactivity, supporting wound healing, while basil EO contributes antimicrobial properties without excessively increasing hydrophobicity.

Roughness

The roughness of materials based on collagen, hydroxyapatite, and essential oils can significantly influence their biological performance, particularly in the context of tissue regeneration and antibacterial applications [24]. For instance, composite materials that combine collagen with hydroxyapatite, like those enhanced with essential oils, can affect cell adhesion, proliferation, and antibacterial activity, depending on the surface roughness. The roughness directly affects the material wettability, interaction with cells, and overall, its applicability for wound dressings [25]. Some studies highlight how the surface roughness of a sample containing essential oils can improve its antibacterial properties and its biological compatibility [26]. The roughness parameters *Ra* (average roughness) and *Rq* (root mean square roughness) are essential for evaluating the texture of a surface, which can influence the adhesion, wettability, and performance of wound dressings.

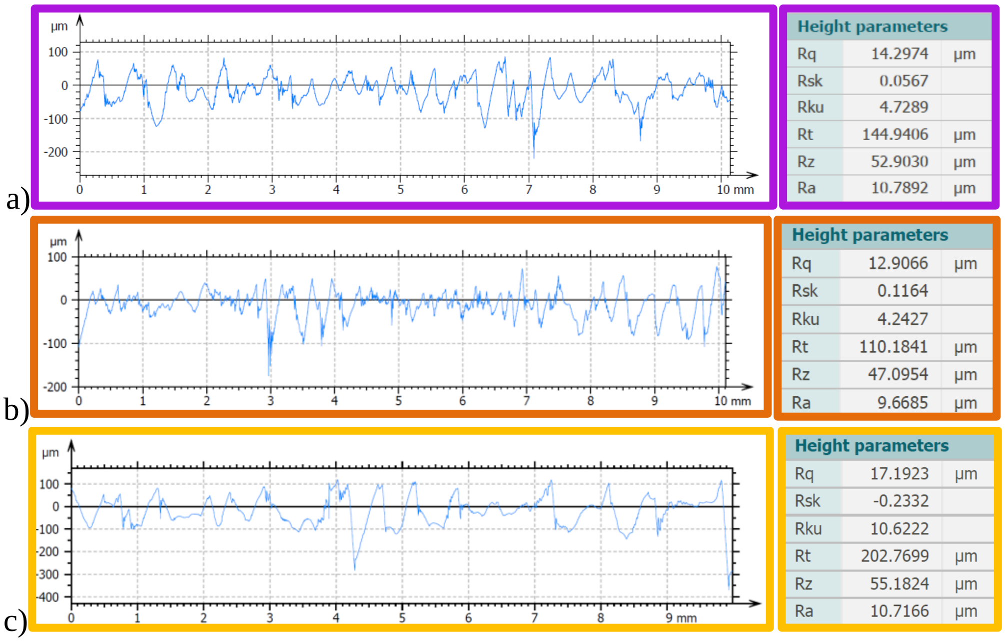

Fig. 6. Roughness profile and high parameters for sample P1(a), sample P2(b), and sample P3(c)

The SEM images demonstrate that porosity significantly influences surface properties. The pore size distribution aligns with the observed contact angle and roughness parameters: sample P3 shows the largest pores, correlating with its higher Rq (17.1923 µm), Rz (55.1824 µm), and Rku (10.6222 µm) values, as well as the lowest contact angle (82.14°). Sample P2 (with 0.005 g HAp and 5µl basil EO) exhibits the smallest pores, which correspond to its lowest roughness (Rq = 12.9066 µm) and the highest contact angle (96.45°), indicating greater hydrophobicity. Sample P1 shows intermediate porosity, matching its intermediate roughness (Rq = 12.9066 µm) and contact angle (87.56°). This trend emphasizes the influence of porosity on surface roughness and wetting behavior. The asymmetry of the surface profile, reflected by Rsk (skewness), indicates pore distribution. The addition of basil EO, which can form a thin and continuous film on the sample surface, contributes to the decrease in roughness compared to samples P1 with 0.005 g HAp and P3 with 0.125 g HAp and 12.5 µl basil EO. The P3 sample shows a similar *Ra* to P1 but a higher *Rq*, indicating a more uneven surface with more pronounced peaks and valleys. The increased *Rq* suggests more significant surface irregularities, probably due to higher concentrations of hydroxyapatite, which contribute to the overall roughness. The roughness (particularly *Rq*) higher than that of sample P1 combined with the relatively higher hydroxyapatite content leads to enhanced hydrophilicity, as seen by the lower contact angle (82.14°). The combination of bioactive hydroxyapatite and basil EO makes the P3 sample effective in managing exudates while offering antimicrobial protection. P3 shows a negative Rsk (- 0.2332) due to the dominance of larger, deeper pores, while P1 (0.0567) and P2 (0.1164) exhibit positive Rsk values, indicating smaller, shallower pores. The peakedness of the surface profile (Rku - kurtosis) is highest in P3 (10.6222), with values more than double those of P1 (4.7289) and P2 (4.2427).

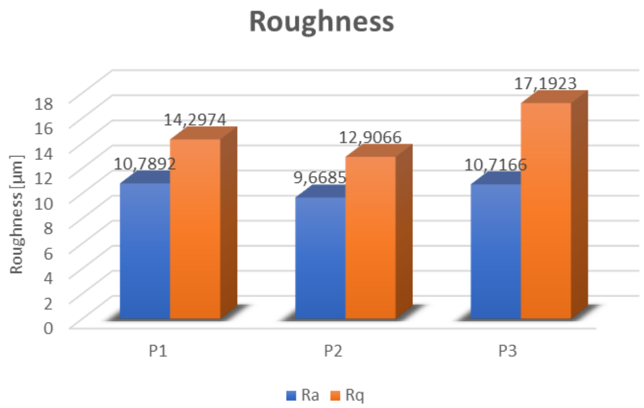

Fig. 7. Comparative results of roughness surface measurements

A smoother and more hydrophobic surface may reduce exudate absorption, potentially limiting wound moisture management. However, the reduced roughness combined with the antimicrobial properties of basil EO could make the P2 sample useful in protecting against infections, especially where fluid absorption is less critical. This is consistent with the pronounced peaks and valleys associated with larger pores in P3. These relationships highlight the influence of the porosity over the surface characteristics and contribute to the observed wettability behavior.

4. Conclusions

Basil essential oil is well recognized for its remarkable pharmacological effects, including antioxidant, anti-inflammatory and antimicrobial properties. Even though it is recognized for its effectiveness in preventing fungal and bacterial infections that impede the wound-healing process, its clinical application is hindered by its limited solubility in aqueous formulations. To remove this inconvenience in this study, compositions with different contents of basil EO were obtained, and oil was introduced in different amounts of hydroxyapatite.

The P1 sample provides hydrophilicity and roughness useful for wound healing, the P3 sample provides hydrophilicity, roughness, and antimicrobial properties useful for wound healing while sample P2 with its smoother, hydrophobic surface enhances antimicrobial properties, providing a diverse set of functionalities for different wound care needs.

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