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Experimental lesion-enriched skin microbiota transplantation by activated charcoal nose pore strips for 'treat to target' superficial pyoderma in dogs

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Abstract: Several bacteria could selectively use substrates among a combination of different carbon sources. Activated carbons exhibit a position for essential medicines and are involved in battling gastrointestinal and dermatological issues by removing toxins from the skin. No study has reported its different mechanism of action against probiotic or pathogenic bacteria. We hypothesized that lesion-enriched skin microbiota transplantation (L-e-smt) using activated charcoal nose pore strips could battle against pyoderma in dogs due to its high adsorption capacity, chemical stability, and mechanical robustness. A total of 16 owned dogs without any prior drug prescription were subjected to clinical and laboratory examinations and diagnosed with superficial pyoderma. They were enrolled and treated with L-e-smt activated charcoal nose pore strips twice weekly. The same animals served as their control, comparing day 0 to day 21. Previously determined scoring for pyoderma among dogs were adopted prior (ranged between 10 to 61) and after (0 to 22) L-e-smt. At the end of the sessions, all 16 dogs were clinically cured without any drug prescription. Based on L-e-smt, the mean superficial pyoderma scoring was 37.10 vs. 9.81 ($p<0.001$) before and post-treatment. The preliminary data from this novel technique could help to improve therapeutic protocols to control superficial pyoderma in dogs. Further experiments are needed to examine microbiota diversity and toxicity of the skin.
Keywords: Microbiota, skin, superficial pyoderma, transplantation.

1. Introduction

The inter-tegumentary system and its foremost significant utilizer, the skin with its entire components, is a significantly zestful organ that maintains the epidermal barrier and modifies external stimuli and alterations. Epidermal and dermal structures, with their relevant components, provide sheltering and reserving of energy (Zwick et al., 2018). The skin additionally resides adjunctions (i.e., hair follicles and glands) involved in several homeostatic issues such as thermoregulation and restoration of wounds (Ito et al., 2005; Lu et al., 2012; Rittie et al., 2013). Sebaceous glands ooze non-polar lipids to prevent water loss, whereas antimicrobial peptides secreted from sweat glands restrict the development of pathogens (Rieg et al., 2006; Schittek et al., 2001). Given that the dermis layer maintains the structural unification of the skin, the epidermis is the initial defensive barrier utilizing uninterrupted proximity with external conditions. Epidermal barrier involves several biological, constructional, and synthesized items pivotal for averting internal infection. On the other hand, disruption of the integumentary barrier because of inflammation, pathological conditions, and bruising could result in cutaneous dysbiosis and elevated infection risk (Howard et al., 2022; Kong et al., 2012; Zeeuwen et al., 2012).

Cutaneous dysbiosis has been described elsewhere (Rodriguez-Campos et al., 2020; Santoro et al., 2024; Sumonja and Kotnik, 2024; Thomsen et al., 2023; Ural, 2022) among dogs. Taking into account the latter data as dysbiosis might be described as switched microbiogeography and functions in the resident microbiome, might occur in skin, the very well-known link "gut-skin axis" could have a pivotal role for integumentary disorders (Ural, 2022; Ural et al., 2024). Hence, the association of pyoderma with skin microbiota composition (Apostolopoulos and Miller, 2023; Older et al., 2020) or skin dysbiosis (Šumonja and Kotnik, 2024; Thomsen et al., 2023) must be precautiously taken into consideration for therapeutical armamentarium.

Given the clinical promise of microbiota transplantation for the intestinal environment, which has been demonstrated in several different research, with well-known curative effects, very few studies have explored (Junca et al., 2022) the clinical usage (Ural et al., 2022; Ural et al., 2023a, 2023b; Ural et al., 2024) and the beneficial effects of skin microbiota transplant (L-e-smt) - moving whole skin bacterial community (Perin et al., 2019). For this purpose, our group developed skin microbiota transplantation with a unique and unmatched/incomparable technique that has never been previously reported (Ural et al., 2022; Ural et al., 2023a, 2023b; Ural et al., 2024). This technique depended on restoring and renewing skin microbiota from heterologous or autologous transplantation of skin microbiota collected by Nivea Skin Refining Clear-Up Strips and transferred to an anatomical location. The success rates were given in detail previously (Ural et al., 2022; Ural et al., 2023a, 2023b; Ural et al., 2024). In the present study, we used Bee Beauty Activated Charcoal Nose Pore Strips as a new alternative material to collect skin microbiota for transplantation. Therefore, this study hypothesizes that using Bee Beauty Activated Charcoal Nose Pore Strips for skin microbiota transplantation can alter the skin microbiota in dogs with pyoderma, leading to an improvement in clinical lesions.

2. Materials and Methods

2.1. Animals

Whole prospective procedures and animal care activities followed the Aydin Adnan Menderes University, Animal Ethic Committee (HADYEK) (Aydın, TURKEY) regulation. This study was approved by the Aydın Adnan Menderes University-HADYEK Local Ethics Committee with approval protocol no: 64583101/2022/132 on December 22, 2022. In this study, solely client-owned dogs (n = 16) were eligible for the study. Dogs were recruited from the Eagean Region and those referred to the Department of Internal Medicine, Veterinary Faculty practice at the University of Aydin Adnan Menderes. Written owner consent forms were obtained from animal owners. Different breeds, age groups, and sexes were enrolled.

2.2. Evidenced based veterinary medicine for relevant laboratory investigations

2.2.1. Clinical scoring for superficial pyoderma

Previously described clinical interpretations of antimicrobials against pyoderma among dogs were adopted (Iwasaki et al., 2008). Thus, i) erythema, ii) papule/pustule, iii) alopecia, iv) crust, and other relevant clinical markers were denoted for clinical appearance. Table 1 describes the severity of the clinical scenario and proportions of pyoderma (Hsiao et al., 2021).

	Pyoderma proportion				
Percentage of total body surface affected	<1%	1%	5%	10%	>10%
Individual grading for dogs from 0 to 4	0=normal	1=mild	2=moderate	3=severe	4=most severe
Clinical interpretation	Final pyoderma score 0 to 112*				

Table 1 – Severity of clinical scenario and proportions of pyoderma. * Upmost scores for superficial pyoderma as 64; 112 for deep pyoderma (adapted from Hsiao et al., 2021).

2.3. Lesion-enriched skin microbiota transplantation (L-e-smt) procedure

L-e-smt was applied in four different sessions. The first step involved shaving the necessary anatomical areas of healthy donor skin (window preparation), which helped remove hair and superficial tissue. Donor selection for heterologous skin microbiota transplantation was carried out by a team of Ph.D. and M.Sc. students. An archive of healthy dogs was maintained, and prior to transplantation, necessary donor recruitment was confirmed by contacting the veterinarian (if needed) at least three hours before the procedure. Four anatomical areas were shaved for the initial L-e-smt. The skin microbiota was then collected using Bee Beauty Activated Charcoal Nose Pore Strips, which were applied to the donor skin moistened with Lactated Ringer's Solution for 10-12 minutes. After removal, the strips were transferred to the peripheral areas of diseased skin for 10-15 minutes. The strips were moistened with a cream formulated with a specific pH. Finally, the strips were removed, and the L-e-smt procedure was completed. Written consent from the animal owners was obtained before the study, and the study was approved by the local ethics committee of Aydın Adnan Menderes University (Approval No: 64583101/2022/132, 22.12.2022).

2.4. Statistical analysis

The mean, median, and 95% confidence interval (CI) values of CADESI-04 scores obtained before and post-treatment were summarized through descriptive statistics. To determine the statistical difference in score changes before and after treatment, the Mann-Whitney U test was employed. In all analyses and graphs, GraphPad Prism software was utilized, and cases even if the p-value was found to be less than 0.05, were considered statistically significant.

3. Results

The study included a total of 16 dogs from a variety of breeds, ages, and sexes. The sample set consisted of breeds such as St. Bernard, Boxer, Terrier, Crossbred, Belgian Malinois, Labrador Retriever, French Bulldog, English breed, Kangal, Golden Retriever, German Shepherd, Chihuahua, and Poodle. The dogs' ages ranged from 2 to 8 years, with a balanced representation of both younger and older dogs. Of the 16 dogs, 9 were males and 7 were female.

3.1. Clinical scoring for superficial pyoderma

Table 2 shows relevant results. Mean and median scoring was available as performed by double-blinded researchers before and after that L-e-smt. All animals gave statistically significant (P = 0.001) differences, regarding 95% CI, superficial scoring results were 23-50 vs. 4-18, before and after L-e-smt treatment, respectively.

Superficial pyoderma scoring	Prior to treatment	Post Treatment	P value
mean	37.10	9.81	
median	40	8	0.001
95% CI	23 - 50	4 - 18	

Table 2 – Superficial pyoderma scoring of treated dogs.

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3.2. Clinical photographs of selected cases

Some selected and striking cases are shown in Figures 1-3. There were no side effects noticed in any dogs enrolled.

Figure 1 – Clinical photograph depicting A) prior to, B-C) during and D) thereafter skin microbiota transplantation with activated charcoal based clear up strips in a dog with muzzle furunculosis and folliculitis.

Figure 2 – Semicircular (A1-2, B1-2) and localized lesion (A3-B3) both on thorax and abdomen of a Pug, 4 years old, prior to (A1-3) and thereafter L-e-smt (B1-3).

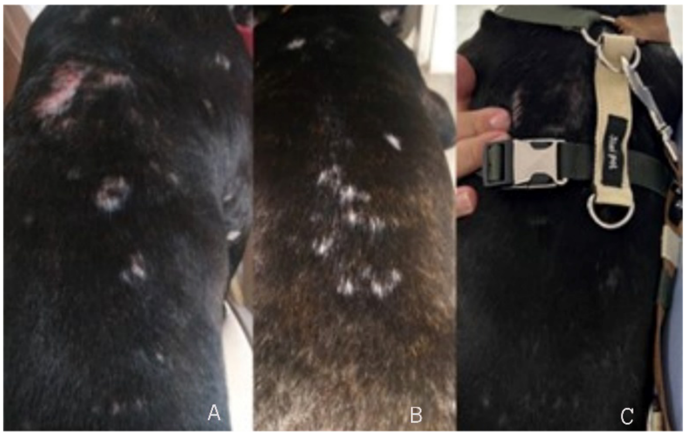

Figure 3 – Dorsolumbar lesions [i.e. alopecia, local crusts and circumscript patches] A) prior to and thereafter B) 1st and C) 2 nd weeks of L-e-smt

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4. Discussion

Thoroughly activated carbon was capable of exhibiting therapeutical efficacy related to biotransformation processes. In this study, the activated charcoal clear-up strips were an attempt to treat pyoderma among dogs. Charcoal has been defined as the carbonic remnant of coconut shells, wood, cellulose, or other relevant manufacturing waste with excessive porosity and surface area. Activating the charcoal through KOH/acids, carbon dioxide, or water vapor influences its porous composition. The resolutely thriving inner surface of active carbon created a highly absorbent character. Taking into account its fine powder (>0.2 mm) availability, it is prone to absorb materials of exactly 20% of its weight. Furthermore, the latter is nontoxic, explaining its usage as a sorbent through pharmacology and cosmetology (Burhacka et al., 2019). Activated carbon detaches several toxins from the digestive system and also the outermost layer of the epidermis (Dalvi and Ademoyero, 1984, Dalvi and McGowan, 1984, Sands et al., 1976).

As a carbon source, Bee Beauty Activated Charcoal Nose Pore Strips were attached to the donor skin in the present study and could thus probably be oxidized and charged negatively. Moreover, to the present author's knowledge, these carbon nose pore strips might facilitate different efficacy for probiotic bacteria and pathogenic ones located on the skin. Cellular respiration has been denoted as one of the central routes by which organisms produce energy. The latter mechanism involves connecting electron donor oxidation (i.e. sugar) for diminishing electron acceptor (i.e. oxygen). Electrons sweep among molecules, recognized as an 'electron transport chain', creating potency and empowering the construction of adenosine triphosphate (Romick et al., 1996, Rivera-Lugo et al., 2022). Respiratory tasks using oxygen or other relevant molecules as electron acceptors are important for the promotion of *Escherichia coli* and *S. enterica* within the intestinal lumen (Rivera-Chávez et al., 2016; Winter et al., 2010; Winter et al., 2013). Several different investigations exhibited the link between respiration with bacterial pathogens for the usage of specific electron donors (i.e. non-fermentable energy sources) within the intestinal lumen (Ali et al., 2014; Faber et al., 2017; Spiga et al., 2017). The vast majority of lactobacilli species with probiotic potential have been well-recognized as oxygen-tolerant anaerobes which did not exhibit a complete electron transport chain (eTc). Probiotic lactobacilli species' aerobic maturation is controlled by the existence of electron acceptors assembling eTc slightest vigorously (Zotta et al., 2014). Occupied oxygen gives rise to the occurrence of reactive oxygen species (ROS) expediting injury against cellular composition (i.e., proteins, lipids, and nucleic acids] (Imlay, 2019). The first-rate bacterial growth promoters in the presence of oxygen are those of strains capable of producing oxidative stress preventive compounds (i.e., catalase and the electron acceptor molecules] (Zotta et al., 2017; Zotta et al., 2018). On the other hand, the exterior side of the activated carbon, when exposed to oxidation, dispensed increased electron density, suggesting elevated concentrations of electrons on its surface. This implies that the oxidized exterior of the activated carbon exhibited a negative charge, which attracted metal ions (Paredes-Doig et al., 2020).

Several different exopolysaccharides are increasingly charged and help water and ion absorption (Chew and Yang, 2016; Lee et al., 2022). Exopolysaccharides, as the vast majority, contained carbohydrate fractions and non-carbohydrate ingredients among which both functional associates elevated the 'negative charge' on them. Moreover, the latter promotes lipophilicity and cooperation with polysaccharides and metal ions (Chew and Yang, 2016). On the other hand, during Gram-positive and Gram-negative bacteria surface assimilation via active carbons, diminished bacterial density in water influences the pH of some compounds (Chu et al., 2013). In prior research 10.3 pH level was detected in all tested active carbon samples (Burchachka et al., 2019). Dissimilarity among compound pH powerfully influences the electrostatic interplay among the active carbon and the adsorbate (Chu et al., 2013). Taking into account the data, beneath the pH situations determined above, carbon material surface pH is negatively charged. However, under diminished pH conditions, the material surface exhibits a positive charge.

It was claimed that activated charcoal exhibited a positive charge even in contact with the *E. coli* and *S. aureus* suspensions (Burchachka et al., 2019). Electrostatic interplay exists among biopolymers loaded with opposite charges (Krasaekoopt et al., 2004). The interplay of biopolymers hangs on conditions such as polysaccharide-protein relations pH and other relevant factors (Salminen & Weiss, 2014). Taking into account, the knowledge that electrostatic interactions occur among opposite charges (Krasaekoopt et al., 2004), both carbon material surface (Burchachka et al., 2019; Chu et al., 2013) and probiotic exopolysaccharides (Chew and Yang, 2016) possesses negative pH charge, may indicate non-interaction among them. However, well-recognized data that activated charcoal might adsorb toxins, spores, and pathogenic microorganisms (Rivera-Utrilla et al., 2001), that the more oxygen species through their exterior boost the increased concentration of adsorptive capacity against Gram-negative/Gram-positive bacteria (Burchachka et al., 2019). All aforementioned mechanisms might be capable of playing a pivotal role in the adsorbance of pathogenic microorganisms but promoting the growth of probiotic bacteria onto the skin, as used in the present study.

One of the limitations of the study is that no medical treatment or antibiotics were used. Antibiotics were avoided due to concerns about microbiota dysbiosis on the skin and gut. Additionally, antibiotics were only considered in cases where sepsis criteria were detected in a clinical scenario. All enrolled dogs exhibited superficial pyoderma without signs of sepsis.

Given confiscation of toxins through the skin via the efficacy of activated charcoal, adds value to their usage within the cosmetic administration (Dalvi and McGowan, 1984; Dalvi and Ademoyero, 1984; Sands et al., 1976). In the present study, we might probably, remove the toxins from the lesional skin, which could thus be related to replacing toxin-secreting pathogenic bacteria.

The scientific era for investigations through field conditions is still in the infancy of establishing the role of bacteria for several alopecia subtypes. *Folliculitis decalvans* has been recognized as the foremost characterized scalp disease involving bacterial contribution of hair follicle inflammation (Constantinou et al., 2021). *S. aureus* was detected in the vast majority of *F. decalvans* cases without medical care in long-duration clinical recovery following antibiotic treatment (Otberg et al., 2008; Annessi, 1998;

Sillani et al., 2010). Previously supposed that *S. aureus* secreted cytotoxic proteins having roles as superantigens stimulating T cells (Powell et al., 1999; Chiarini et al., 2008). The existence of *S. aureus* was inflated in the scalp skin of *F. decalvans* cases and superficial occurrence on lesional skin was elevated by the probability of subepidermal colonization (Matard et al., 2020). Similarly, given the data that an overall elevation in *Staphylococcus spp.* abundance was detected among dogs with superficial pyoderma (Older et al., 2020). The use of Bee Beauty Activated Charcoal Nose Pore Strips for battling pyoderma could be able to adsorb pathogenic bacteria onto the skin. The removed pathogenic bacteria (substitution with probiotics from the donor to the recipient) could have hastened clinical recovery, as well.

5. Conclusion

In this study, we demonstrated the potential of L-e-smt using activated charcoal nose pore strips as an innovative treatment for superficial pyoderma in dogs. The high adsorption capacity of activated charcoal allowed for the removal of toxins and pathogenic microorganisms from the skin, potentially promoting the growth of beneficial probiotic bacteria. Clinical improvements were observed in all enrolled dogs, with significant reductions in pyoderma scores, without the use of antibiotics or other medical treatments. This novel technique offers a promising alternative for treating pyoderma by leveraging the adsorption properties of activated charcoal to balance the skin microbiota. However, further research is required to explore the long-term efficacy, microbiota diversity, and potential side effects of this treatment. The findings of this study contribute to the evolving field of microbiota transplantation and highlight its potential therapeutic applications for dermatological conditions in veterinary medicine.

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