An optimized mouse model of Staphylococcus aureus infected diabetic ulcers

Ana Isabel MENDES¹,², Maria João PEIXOTO¹,², Alexandra Pinto MARQUES²,³, Jorge PEDROSA¹,² and Alexandra Gabriel FRAGA¹,²*

Abstract

Objective: Diabetic foot infection (DFI) represents a major healthcare burden, for which treatment is challenging owing to the pathophysiological alterations intrinsic to diabetes and the alarming increase of antimicrobial resistance. Novel therapies targeting DFI are therefore a pressing research need for which proper models of disease are required.

Results: Here, we present an optimized diabetic mouse model of methicillin-resistant Staphylococcus aureus (MRSA)-infected wounds, that resemble key features of DFI, such as pathogen invasion through wound bed and surrounding tissue, necrosis, persistent inflammation and impaired wound healing. Thus, in a time-efficient manner and using simple techniques, this model represents a suitable approach for studying emerging therapies targeting DFI caused by MRSA.

Keywords: Chronic wounds, Diabetic mouse model, Impaired wound healing, Inflammation, MRSA infection

Introduction

Management of chronic ulcerative wounds is a critical worldwide healthcare challenge, associated with a high risk of morbidity and mortality [1, 2]. Diabetes is an important predisposition factor for skin ulceration, particularly on the foot, which is often complicated by infection [3, 4]. Several pathogens can be found in diabetic foot infection (DFI), but Staphylococcus aureus is the most common [5–7]. S. aureus typically forms biofilms, evading the activity of both the host immune system and antibiotics, hampering current treatment strategies [6, 8–10]. The lack of effective treatment is further aggravated, when considering the alarming increase of methicillin-resistant S. aureus (MRSA) prevalence in DFI [7, 8]. Thus, the development of new therapeutic strategies for DFI is critical, for which appropriate and reliable models are urgently required. In this regard, this study developed an optimized protocol for generating standardized MRSA-infected wounds in a diabetic mouse model of impaired healing that mimics the main hallmark features of DFI, including continuous necrosis and inflammation associated with invasive infection of the wound bed, and can be easily employed to test novel therapies targeting DFI.

Main text

Methods

Animal experimentation was performed at the Life and Health Sciences Research Institute at the University of Minho, in accordance with the Directive 2010/63/EU, and approved by Institutional Animal Care and Use Committee of University of Minho. 8–12-week-old male C57BL/6 mice (Charles River Laboratories) were housed under specific pathogen-free condition with food and water ad libitum and acclimatized for 1 week before the experiment. Mice (n = 14) were equally and randomly divided in two groups corresponding to established endpoints of 2- and 9-days post-infection (dpi). Randomization was performed using randomize function of...
Microsoft® Excel®, Humane endpoints were followed as described on Additional file 1: Table S1.

Diabetes induction
Type 1 diabetes mellitus (T1DM) was chemically induced, as previously described [11], administering 50 mg/kg of streptozotocin (STZ) (Merck KGaA, Germany) for 5 consecutive days. Blood glucose levels were measured 9 days after STZ treatment, using a monitor glucose device. Levels of blood glucose higher than 150 mg/dL were considered hyperglycemic. Mice were observed for signs of polydipsia and polyuria throughout the experimental period.

Dorsal fur depilation
On the day before surgery, dorsal fur of mice was shaved, using a hair clipper followed by depilatory cream for 1 min. Cream was further removed by wiping the skin with cotton soaked in warm water.

Inoculation of polycarbonate membranes
Briefly, 0.2 µm pore size polycarbonate membranes (Merck KGaA, Germany) were cut in 5-mm diameter discs, sterilized on both sides by UV light for 30 min and then placed on mannitol salt agar (MSA). A bacterial suspension of 10⁸ colony forming units (CFU)/ml of *S. aureus* Rosenbach (ATCC BAA 2313) was prepared using isolated colonies previously grown on MSA, that were resuspended in saline and further inoculated on the membranes (10⁵ CFU/membrane). Inoculated membranes were incubated overnight at 37 ºC to grow a biofilm (attaining approximately 10⁹ CFU/membrane).

Excisional wounding and infection
Mice were intraperitoneally injected with anesthetics (75 mg/kg ketamine and 1 mg/kg medetomidine) and analgesia (0.1 mg/kg buprenorphine). Two symmetrical full-thickness excisional wounds were created using a 75 mg/kg of streptozotocin (STZ) (Merck KGaA, Germany) for 5 consecutive days. Blood glucose levels were measured 9 days after STZ treatment, using a monitor glucose device. Levels of blood glucose higher than 150 mg/dL were considered hyperglycemic. Mice were observed for signs of polydipsia and polyuria throughout the experimental period.

Excisional wounds were covered with Durapore™ self-adhering bandage (3 M, USA) (Fig. 1C). The implementation of diabetes was achieved with STZ by damaging insulin producing β cells of pancreatic islets. All animals were considered diabetic, showing blood glucose levels of 312.4 ± 90.8 mg/dL (Fig. 2A), excessive water consumption and urine production.

Regarding the infection of inflicted wounds, a biofilm covering the wound bed was clearly identified both macroscopically (Fig. 1E) and microscopically (Fig. 2C). The biofilm formation was identical in both wounds and among animals. At 2-dpi, wounds bacterial burden reached a mean log₁₀ CFU of 8.32 that significantly increased to 9.14 at 9-dpi (Fig. 2B). Histological analysis revealed that MRSA was present not
only on the wound surface but was also able to spread into the surrounding non-wounded deep tissue. Furthermore, infection led to necrosis and instigated the infiltration of inflammatory cells in the vicinity of the areas of bacterial accumulation (Fig. 2C). Wounds remained open over the experimental period, without signs of fibroplasia or granulation tissue formation, and revealed an identical pattern of inflammation and infection along different sections of wound area. It is noteworthy that two animals from the 9-dpi experimental group succumbed before the established endpoint.
Discussion

This study reports an optimized protocol to obtain standardized MRSA-infected chronic wounds in a diabetic mouse model that mimics human DFI pathophysiology. Not only did mice reveal signs of T1DM, such as hyperglycemia, polydipsia and polyuria [11], but experimentally inflicted wounds in diabetic mice showed an effective infection by MRSA that triggered continuous necrosis and infiltration of immune cells. This arrested state of necrosis associated with an inflammatory microenvironment hindered healing progression, representing key hallmarks of wound chronicity [2, 4]. Ultimately, this model depicts critical features of the DFI microenvironment that previous animal models of infected wounds failed to validate, including: i) the ability of MRSA to penetrate and spread within the wound bed, rather than being restricted to the scab [12–15]; ii) minimizing primary skin contraction that naturally occurs in rodents, which allows a healing mechanism by re-epithelization [12, 14, 16, 17]; and iii) control over the causative agent and dose of infection, circumventing the lack of standardization observed in models of naturally infected wounds.

Fig. 2  Representative outcomes of the protocol of MRSA-infected wounds in a diabetic mouse model: A Blood glucose levels (mg/dL) after 9 days of STZ treatment; B bacterial burden of wounds at 2- and 9-dpi; C H&E and Gram-stained sections of wounds at 2- and 9-dpi (b: bacteria; i: inflammatory immune cells; n: necrosis)
wounds that depend on housing conditions, source of animal colonies and host skin microbiome [18].

All techniques herein employed were simple, easily executed and optimized in terms of timing and number of interventions to minimize animal morbidity and mortality, although they should be expected, especially at later timepoints of infection. Importantly, the behavior and mobility of mice were not significantly impacted by dressings, that remained in place and were not removed by mice, even though they were caged in group.

Overall, this model offers a simple and suitable approach for studying emerging technologies of topical application for the treatment of MRSA-infected chronic wounds, that are difficult to test in the available animal models. Ultimately, it can be adapted to other diabetic animal models or to other pathophysiological conditions.

Limitations
Wound infection with a single bacterial specie constitutes a limitation of this study. It would be relevant to further apply this protocol using polymicrobial biofilms to evaluate the interspecies relationship on the wound healing process.

Abbreviations
DFI: Diabetic foot infection; MRSA: Methicillin-resistant Staphylococcus aureus; Dpi: Days post-infection; T1 DM: Type 1 diabetes mellitus; STZ: Streptozotocin; MSA: Mannitol salt agar; CFU: Colony forming units; H&E: Hematoxylin and Eosin.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13104-022-06170-5.

Additional file 1: Table S1. Animal welfare scoresheet and humane endpoints.

Acknowledgements
Not applicable.

Author contributions
AM: conceptualization, data collection and interpretation, writing – original draft preparation, writing—review and editing. MJ: data collection, writing—review and editing, APM and JP: conceptualization, data interpretation, writing—review and editing. AGF: Conceptualization, data collection and interpretation, writing—review and editing. All authors read and approved the final manuscript.

Funding
Financial support was provided to AM through the PhD grant UMINHO/BD/53/2017 supported by the project NORTE-08-5369-FSE-00041, financed by Operational Program NORTE2020 and co-financed by the European Social Fund. Financial support was also provided by National funds, through the Foundation for Science and Technology (FCT)-project UIDB/50026/2020 and UIDP/50026/2020.

Availability of data and materials
The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethical approval and consent to participate
Animal experimentation was performed in accordance with the Directive 2010/63/EU and approved by Institutional Animal Care and Use Committee of University of Minho.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 School of Medicine, Life and Health Sciences Research Institute (ICVS), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. 2 ICVS/3B’s – PT Government Associate Laboratory, Braga, Guimarães, Portugal. 3 3B’s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradable and Biomimetics, Headquarters of the European Institute of Excellence On Tissue Engineering and Regenerative Medicine, University of Minho, AvePark, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal.

Received: 10 May 2022 Accepted: 10 August 2022

Published online: 07 September 2022

References
1. Avitha E, Yeghiazaryan K, Golubnitschaja O. Impaired wound healing: facts and hypotheses for multi-professional considerations in predictive, preventive and personalised medicine. EPMA J. 2017;8(1):23–33. https://doi.org/10.1007/s13167-017-0081-y.
2. Atkin L, Bucko Z, Montero EC, et al. Implementing TIMERS: the race against hard-to-heal wounds. J Wound Care. 2019;28(3):55–50. https://doi.org/10.12968/jowc.2019.28.Sup3a.S1.
3. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med. 2017;376(24):2367–75. https://doi.org/10.1056/NEJMrA1615439.
4. Patel S, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomed Pharmacother. 2019. https://doi.org/10.1016/j.biopha.2019.108615.
5. Lipsky BA, Aragon-Sanchez J, Diggie M, et al. IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. Diabetes Metab Res Rev. 2016;32:45–74. https://doi.org/10.1002/dmrr.2699.
6. Smith K, Collier A, Townsend EM, et al. One step closer to understanding the role of bacteria in diabetic foot ulcers: characterising the microbiome of ulcers. BMC Microbiol. 2016;16(12):54. https://doi.org/10.1186/s12866-016-0665-z.
7. Silva V, Almeida F, Carvalho JA, et al. Emergence of community-acquired methicillin-resistant staphylococcus aureus EMRSA-15 clone as the predominant cause of diabetic foot ulcer infections in Portugal. Eur J Clin Microbiol Infect Dis. 2020;39(1):179–86. https://doi.org/10.1007/s10096-019-03709-6.
8. Negut I, Grumenezcu V, Grumenezcu AM. Treatment strategies for infected wounds. Molecules. 2018;23(9):2392. https://doi.org/10.3390/molecules23092392.
9. Mottola C, Matias CS, Mendes JJ, et al. Susceptibility patterns of Staphylococcus aureus biofilms in diabetic foot infections. BMC Microbiol. 2016. https://doi.org/10.1186/s12866-016-0737-0.
10. Zhao G, Usui ML, Lippman SI, et al. Biofilms and inflammation in chronic wounds. Adv Wound Care. 2013;2(7):389–99. https://doi.org/10.1089/wound.2012.0381.
11. Wu KK, Huan Y. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2008. https://doi.org/10.1002/0471141755.ph0547/v40.
12. Silva V, Peirone C, Capita R, et al. Topical application of ozonated oils for the treatment of MRSA skin infection in an animal model of infected ulcer. Biology. 2021;10(5):372. https://doi.org/10.3390/biology10050372.
13. Shi CM, Nakao H, Yamazaki M, Tsuboi R, Ogawa H. Mixture of sugar and povidone-iodine stimulates healing of MRSA-infected skin ulcers on db/db mice. Arch Dermatol Res. 2007;299(9):449–56. https://doi.org/10.1007/s00403-007-0776-3.
14. Guo Y, Ramos RI, Cho JS, Donegan NP, Cheung AL, Miller LS. In Vivo bioluminescence imaging to evaluate systemic and topical antibiotics against community-acquired methicillin-resistant staphylococcus aureus-infected skin wounds in mice. Antimicrob Agents Chemother. 2013;57(2):855–63. https://doi.org/10.1128/aac.01003-12.
15. Zhao G, Hochwalt PC, Usui ML, et al. Delayed wound healing in diabetic (db/db) mice with Pseudomonas aeruginosa biofilm challenge: a model for the study of chronic wounds. Wound Repair Regen. 2010;18(5):467–77. https://doi.org/10.1111/j.1524-475X.2010.00608.x.
16. He H, Xia DL, Chen YP, et al. Evaluation of a two-stage antibacterial hydrogel dressing for healing in an infected diabetic wound. J Biomed Mater Res B Appl Biomater. 2017;105(7):1808–17. https://doi.org/10.1002/jbm.b.33543.
17. Tong CY, Zhong XH, Yang YJ, et al. Pb@PDA@Ag nanosystem for synergistically eradicating MRSA and accelerating diabetic wound healing assisted with laser irradiation. Biomaterials. 2020;243(14): 119936. https://doi.org/10.1016/j.biomaterials.2020.119936.
18. Kim JH, Martins-Green M. Protocol to create chronic wounds in diabetic mice. J Vis Exp. 2019. https://doi.org/10.3791/57656.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.