

The impact of chemotherapy on the host microbiota in the context of oral and gastrointestinal mucositis

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SUMMARY

In this PhD thesis, we investigated the impact of chemotherapy on the microbiota in the context of mucositis by using different experimental set-ups. Using bacterial monocultures, we showed that exposure to 5-fluorouracil at physiologically relevant concentrations differentially impacts oral microorganisms. Despite this difference in microbial sensitivity to 5-fluorouracil in pure cultures, we showed that the impact of 5-fluorouracil, as well as irinotecan, towards highly diverse gastrointestinal microbial populations is only marginal. These findings were generated with two different model systems that exclude host cells and this led us to conclude that the host is crucial in the establishment of chemotherapy-induced shifts in microbial composition and functionality. The next step in our research entailed the use of an *in vitro* wound healing model, where we demonstrated that the presence of microbiota negatively impacts the wound healing capacity of damaged oral epithelial cells. This indicates that microbial presence can delay the recovery from mucositis. Yet, we also found that microbial composition, which is for instance disturbed in patients receiving cancer therapy, is an additional determinant of aggravated wound healing. We further substantiated this conclusion with an *in vivo* longitudinal monitoring study of paediatric patients treated for haematological malignancies. While shifts in the oral microbial community during and following chemotherapy were mostly patient-specific, clear associations were made with the use of systemic antibiotics and antibacterial mouth rinses, which create microbial dysbiosis. In view of these findings we propose that the preventive use of antimicrobials needs careful consideration given the profound impact on the microbiome and subsequent consequence for the host.

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INTRODUCTION

Oral and gastrointestinal mucositis significantly impact the quality of life of cancer patients. Mucositis may lead to a reduction or delay of cancer treatment and unfortunately good treatment options are elusive.¹ A mounting body of evidence suggests a key role for the microbiota in mucositis development.²⁻⁵ However, the underlying mechanisms remain unclear. Microbial shifts have been observed following chemotherapy in both clinical and animal studies.⁶⁻⁹ However, it is not clear whether chemotherapy directly induces microbial shifts or if chemotherapy causes a disturbed host environment inducing microbial changes. In the *in vitro* experiments in this thesis, we focused on two commonly used chemo-

therapeutic agents with high incidence of mucositis: 5-fluorouracil (5-FU) and irinotecan (SN-38).

5-FU SENSITIVITY VARIES AMONG ORAL MICRO-ORGANISMS

Firstly, the direct effect of physiologically relevant concentrations of 5-FU on the viability and growth of oral bacterial monocultures was investigated.¹⁰ 5-FU sensitivity varied among the tested oral species. *Klebsiella oxytoca*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus oralis*, *Pseudomonas aeruginosa* and *Lactobacillus salivarius* appeared to be highly resistant to all tested concentrations (0.1-50 μ M). In contrast, *Lactobacillus oris*, *Lactobacillus plantarum*, *Streptococcus pyo-*

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genes, *Fusobacterium nucleatum* and *Neisseria mucosa* showed a significant reduction in growth and viability starting from very low concentrations (0.2–3.1 μM). We also provided evidence that dihydropyrimidine dehydrogenase, an enzyme involved in 5-FU resistance in humans, is not involved in the 5-FU resistance of the selected species.

5-FU AND IRINOTECAN HAVE LIMITED IMPACT ON THE COLON MICROBIOME

To assess the direct impact of chemotherapeutic agents on a complex microbial ecosystem, we used the M-SHIME[®], an *in vitro* mucosal simulator of the human intestinal microbial ecosystem.¹¹ The direct impact of 5-FU and SN-38 on the luminal and mucosal gut microbiota from several human donors was investigated.¹² At a dose of 10 μM , 5-FU impacted the functionality and composition of the colon microbiota to a minor extent. Similarly, a daily dose of 10 μM SN-38 did not cause significant changes in the functionality or microbiome composition. As our mucosal model does not include a host compartment, we therefore assume that the changed microbiome observed *in vivo* is primarily induced by an altered host environment upon chemotherapeutic treatment.

ORAL MICROBIOTA REDUCE WOUND HEALING CAPACITY OF ORAL EPITHELIAL CELLS, IRRESPECTIVE OF THE PRESENCE OF 5-FU

The effect of 5-FU was assessed in an *in vitro* co-culture model that consists of an epithelial cell layer and a biofilm derived from oral microbiota from different oral regions (saliva, buccal and tongue swabs) and donors (healthy individuals and patients suffering from mucositis).¹³ Oral microbiota reduced wound healing capacity of epithelial cells with higher bacterial cell counts linked to lower wound healing capacity in healthy individuals. However, for patients suffering from mucositis wound healing was more related to microbial composition, rather than microbial load. Indeed, these oral samples were characterised by a disturbed microbial community and higher abundances of pathogenic genera. However, no major impact of 5-FU on wound healing capacity or the composition of the microbiome was seen. These results emphasise the importance of controlling bacterial load by oral hygiene for proper oral wound healing in healthy individuals. However, extra measures besides oral hygiene might be necessary to assure a good wound healing during mucositis.

LONGITUDINAL ANALYSIS OF ORAL MICROBIOTA IN PAEDIATRIC PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

To assess the impact of chemotherapy *in vivo*, a longitudinal

study of the oral microbiota from five paediatric patients, treated with chemotherapy for haematological malignancies (three Burkitt lymphoma, one Burkitt leukaemia and one acute myeloid leukaemia), was performed. Microbial community composition analysis by 16S rRNA gene based Illumina sequencing showed that large microbial dynamics were present throughout therapy in all patients, however shifts were patient-specific. Mucositis lesions were highly dominated by *Streptococcus*, but also by more pathogenic genera such as *Aggregatibacter*, *Enterococcus* and *Fusobacterium*. Surprisingly, chemotherapy and mucositis had only a minor effect on microbial community composition, whereas one of the major confounding factors of our study was the use of systemic antibiotics as it majorly affected both microbial composition and diversity. Other confounding factors were sample type and sampling period, but also the use of antibacterial mouth rinse with chlorhexidine, neutropenia and inflammation. While the overall community composition seemed to return to its initial composition at least one month after therapy, a sustained impact towards a lower diversity was noted. This indicates the importance of long-term follow-up of oral health and good oral hygiene for these patients.

CONCLUSIONS

In conclusion, this PhD research demonstrated that the direct effect of chemotherapy on the oral and gut microbiome is limited, but that the chemotherapy-disturbed host environment may largely impact the host microbiota. A low bacterial load may improve wound healing capacity and reduce risk of infection. Mechanical removal of oral microbiota is preferred, as antimicrobial rinses may cause microbial shifts leading to dysbiosis. However, long-term follow-up and extra measures are needed to assess a fast and full recovery of both host mucosa and microbiota in chemotherapy-induced mucositis.

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KEY MESSAGES FOR CLINICAL PRACTICE

- 1** Although the direct impact of chemotherapy on the oral and gastrointestinal microbiota is limited, chemotherapy will have an indirect impact on the microbiome via the chemotherapy-disturbed host environment.
- 2** Oral hygiene is very important to improve wound healing. Yet, for patients suffering from mucositis, extra measures besides good oral hygiene may be needed.
- 3** (Prophylactic) antibiotics should be used very carefully as they also highly impact the oral microbiome.
- 4** Antibacterial mouth rinses shift the oral microbiome and are therefore not recommended for prevention or treatment of oral mucositis.
- 5** Long-term follow-up of oral hygiene is important following oral mucositis as there is only partial recovery of the oral microbiome in the first months.

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