Chronotherapy in Oral Health and Post-Operative Recovery

Mohammad Abusamak, BDS

Ph.D. in Oral Health Sciences

Faculty of Dental Medicine and Oral Health Sciences

McGill University, Montreal

April 2024

Supervisor: Prof. Belinda Nicolau

Co-supervisor: Prof. Faleh Tamimi

A thesis submitted to McGill University in partial fulfillment of the requirements of the Doctoral of Philosophy Degree

Dedication

This thesis is dedicated to all men, women and children of Gaza and Palestine.

Preface

My thesis follows a manuscript-based format according to the Graduate and Postdoctoral Studies of McGill University. The introduction, literature review, and methods chapters are followed by three manuscripts demonstrating the findings of my project. All chapters were written by me (Mohammad Abusamak) under the supervision of Prof. Belinda Nicolau and Prof. Faleh Tamimi.

Table of Contents

List of figures

List of Tables

ABSTRACT

Background: In humans, circadian rhythms (~ 24h) control all major organ systems, thus orchestrating nearly all vital aspects of our physiology and metabolism, including processes very important to dentistry, such as bone healing, the immune response, inflammation, and nociception. Chronotherapy, the time of administration tailored to the circadian rhythm, is an emerging field aiming to improve therapeutic efficacy and decrease adverse effects in many medical fields. Oral and craniofacial health is closely intertwined with general well-being, and dentistry is a multidimensional discipline that comprises a wide range of pathologies, surgical and non-surgical interventions, and rehabilitations that could greatly benefit from the applications of chronotherapy. **Methods:** A combination of research methods was used to investigate chronotherapy in oral health and post-operative recovery. First, a scoping review was conducted to systematically map the research carried out regarding chronotherapy in dentistry and identify any existing gaps in knowledge. Second, two randomized controlled trials (Parallel & Cross-over) were carried out to investigate the effect of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) chronotherapy on post-operative pain management and swelling in an impacted third molar extraction model. **Results:** The scoping review showed that chrono-radiotherapy and chrono-chemotherapy reduced treatment side effects and improved therapeutic response, leading to higher survival rates in cancer patients. In addition, profound and prolonged local anesthesia could be achieved when injected in the evening. The randomized controlled trials showed that there were no significant differences between the chronotherapy and control groups of the study in terms of postoperative pain scores or any other healing indicator. **Conclusion:** Although the overall quality of the included studies in the scoping review was low, chronotherapy applications in dentistry seem to have favourable outcomes, especially in head and neck cancer treatments. Further, postoperative pain follows a

circadian rhythm. Moreover, the night administration of ibuprofen might not provide any significant benefits in terms of pain management and control of inflammation, and two doses during the day could only be sufficient for pain management after surgical interventions.

ABRÉGÉ

Contexte : Chez l'homme, les rythmes circadiens (~ 24h) contrôlent tous les principaux systèmes organiques, orchestrant ainsi presque tous les aspects vitaux de notre physiologie et de notre métabolisme, y compris des processus très importants pour la dentisterie, tels que la cicatrisation osseuse, la réponse immunitaire, l'inflammation et la nociception. La chronothérapie, c'est-à-dire l'adaptation de l'heure d'administration au rythme circadien, est un domaine émergent qui vise à améliorer l'efficacité thérapeutique et à réduire les effets indésirables dans de nombreux domaines médicaux. La santé bucco-dentaire et cranio-faciale est étroitement liée au bien-être général, et l'odontologie est une discipline multidimensionnelle qui comprend un large éventail de pathologies, d'interventions chirurgicales et non chirurgicales, et de rééducations qui pourraient grandement bénéficier des applications de la chronothérapie. **Méthodes :** Une combinaison de méthodes de recherche a été utilisée pour étudier la chronothérapie dans le domaine de la santé bucco-dentaire et de la récupération post-opératoire. Tout d'abord, un examen approfondi a été effectué pour recenser systématiquement les recherches menées sur la chronothérapie en dentisterie et identifier les lacunes existantes dans les connaissances. Ensuite, deux essais contrôlés randomisés (parallèles et croisés) ont été menés pour étudier l'effet de la chronothérapie par anti-inflammatoires non stéroïdiens (AINS) sur la gestion de la douleur et de l'enflure postopératoires dans un modèle d'extraction d'une troisième molaire incluse. **Résultats :** L'étude exploratoire a montré que la chrono-radiothérapie et la chrono-chimiothérapie réduisaient les effets secondaires du traitement et amélioraient la réponse thérapeutique, conduisant à des taux de survie plus élevés chez les patients atteints de cancer. En outre, une anesthésie locale profonde et prolongée peut être obtenue en cas d'injection le soir. Les essais contrôlés randomisés ont montré qu'il n'y avait pas de différences significatives entre les groupes de chronothérapie et de contrôle de l'étude en termes

de scores de douleur postopératoire ou de tout autre indicateur de guérison. **Conclusion :** Bien que la qualité globale des études incluses dans la revue exploratoire soit faible, les applications de la chronothérapie en dentisterie semblent avoir des résultats favorables, en particulier dans les traitements du cancer de la tête et du cou. En outre, la douleur postopératoire suit un rythme circadien. En outre, l'administration nocturne d'ibuprofène pourrait ne pas apporter d'avantages significatifs en termes de gestion de la douleur et de contrôle de l'inflammation, et deux doses pendant la journée pourraient être suffisantes pour la gestion de la douleur après des interventions chirurgicales.

ACKNOWLEDGMENT

First and foremost, all praise is due to Allah. I thank Allah the most gracious the most merciful for giving me the energy, power, and perseverance to finally reach the finish line of this long journey.

I wish then to express my eternal gratitude to, both my supervisor and co-supervisor, Prof. Belinda Nicolau and Prof. Faleh Tamimi for their endless support, encouragement, and excellent mentorship. I have learned so much from you two. You always believed in me, were on my side, and dedicated your efforts and time to guide me through this journey. You greatly shaped the person who I am today. For this and many other things, thank you!

I also wish to thank the members of my advisory committee Dr. Monzur Murshed and Dr. Sreenath Madathil, for their continuous support and guidance throughout my studies. I learned a lot from you, thank you.

My very special loving thanks to my parents, Omar and Isra, my brothers, Abdelaziz, Abdullah, and Ibrahim, and my lovely sister, Mariam for their unconditional love and support. I am equally lucky and proud to be part of this family. Biggest thank you from the bottom of my heart.

To my soulmate, my wife Aseel; You are the blessing of my life, and you are always loving and supportive endlessly. Without you, my journey would have been colorless. Thank you my love! I would also like to thank my in-laws, Abd Alhameed and Areej, for their support and care.

My sincere appreciation to the Faculty of Dental Medicine and Oral Health Sciences at McGill University, including faculty members, staff, students, friends, and colleagues. Special thanks to my best friend Mohammad Al-Tamimi for his support and care. I am grateful for every minute we spent in Montreal together. Also, I would like to extend my appreciation to the Muslim Association of Canada – South chapter & Vancouver chapter for their spiritual guidance and support.

Last but not least, I wish to acknowledge the funding agencies of my project, the Canadian Institutes of Health Research (CIHR) and Réseau de recherche en santé buccodentaire et osseuse (RSBO). Also, I would like to thank the Ontario Student Assistance Program (OSAP) for their financial support as well.

CONTRIBUTIONS OF CO-AUTHORS

Manuscript I:

Chronotherapy in Dentistry: A Scoping Review

Published in the Chronobiology International Journal

Mohammad Abusamak, Ph.D. Candidate, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Project conception, performed all steps of the scoping review (searching the literature, selection of studies, data extraction, and quality assessment), interpreted the results, and wrote the manuscript.

Mohammad Al-Tamimi, MSc Candidate in the Faculty of Dentistry, University of Toronto, Ontario, Canada: Participated in the screening, selection process, data extraction, and manuscript reviewing.

Haider Al-Waeli, Assistant Professor, Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia, Canada: Participated in data interpretation and manuscript reviewing.

Kawkab Tahboub & Wenji Cai, Ph.D. students at the Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Participated in quality assessment and manuscript review.

Martin Morris, Full-time librarian, Schulich Library of Physical Sciences, Life Sciences and Engineering, McGill University, Montreal, Quebec, Canada, designed the search strategies for four databases and participated in writing the methodology.

Faleh Tamimi, Professor, College of Dental Medicine, QU Health, Qatar University, Doha, Qatar: Supervised the conception of the manuscript and all steps of the scoping review, including data interpretation and writing and reviewing the manuscript.

Belinda Nicolau, Professor, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Supervised the conception of the manuscript and all steps of the scoping review, including data interpretation, writing and reviewing the manuscript.

Manuscript II:

NSAID chronotherapy after impacted third molar extraction: a randomized controlled trial

Published in the Oral and Maxillofacial Surgery Journal

Zaid Tamimi, Associate Professor, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan: Performed the surgical procedures, participated in the grant submission, participated in the protocol registration, and manuscript reviewed.

Mohammad Abusamak, Ph.D. Candidate, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Data management, visualization and analyses, wrote and revised the manuscript.

Haider Al-Waeli, Assistant Professor, Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia, Canada: Provided the idea for the study, established the hypothesis, constructed the database, submitted the protocol to clinicaltrial.gov and revised the manuscript.

Mohammad Al-Tamimi, MSc Candidate in the Faculty of Dentistry, University of Toronto, Ontario, Canada: Participated in the recruitment of the patients, filled out patient's records and reviewed the manuscript.

Rola Al Habashneh, Professor, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan: Reviewed the manuscript.

Mohammad Ghanim, Maxillofacial resident, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan: Participated in the recruitment of the patients, filled patients' records and reviewed the manuscript.

Mohammed Al-Nusair, Medical Student**,** Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan: Reviewed the manuscript.

Faleh Tamimi, Professor, College of Dental Medicine, QU Health, Qatar University, Doha, Qatar: Supervised the conception of the manuscript, conceived the study, designed the trial, and edited and reviewed the manuscript.

Belinda Nicolau, Professor, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Supervised the conception of the manuscript, conceived the study, designed the trial, and edited and reviewed the manuscript.

Manuscript III:

Effect of time-dependent ibuprofen administration on the post-operatory after impacted third molar extraction: a cross-over randomized controlled trial

Published in the Oral and Maxillofacial Surgery Journal

Fabián Pérez-González, Ph.D. Candidate, Faculty of Dentistry, Department of Dental Clinical Specialties, University Complutense of Madrid, Plaza Ramón y Cajal S/N, 28040, Madrid, Spain: Participated in the recruitment of patients, performed the surgical procedures, participated in the protocol registration, wrote and reviewed the manuscript.

Mohammad Abusamak, Ph.D. Candidate, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Project conception, study design, data management, visualization and analyses, wrote and reviewed the manuscript.

Luis Miguel Sáez-Alcaide, Faculty of Dentistry, Department of Dental Clinical Specialties, University Complutense of Madrid, Plaza Ramón y Cajal S/N, 28040, Madrid, Spain: Reviewed the manuscript.

Jesus Torres García-Denche, Faculty of Dentistry, Department of Dental Clinical Specialties, University Complutense of Madrid, Plaza Ramón y Cajal S/N, 28040, Madrid, Spain: Supervised patients recruitment, surgical procedures, and reviewed the manuscript.

Faleh Tamimi, Professor, College of Dental Medicine, QU Health, Qatar University, Doha, Qatar: Supervised the conception of the manuscript, conceived the study, designed the trial, and edited and reviewed the manuscript.

STATEMENT OF ORGINALITY

The research presented in this thesis is original, and my work collectively contributes original knowledge by presenting a comprehensive review summarizing the application of chronotherapy in dentistry and investigating the effect of chronotherapy on post-operative recovery in a clinical setting.

In manuscript I, I performed a scoping review that systematically mapped the available evidence pertaining to the application of chronotherapy in dentistry, marking the first comprehensive review in this field. In manuscript II and III, I conducted two randomized controlled trials with two different study designs (Parallel and Cross-over) to evaluate Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) chronotherapy following third molar extraction.

1 Chapter 1: Introduction

In humans, circadian rhythms (~ 24h) control all major organ systems, thus orchestrating nearly all vital aspects of our physiology and metabolism, including processes very important to dentistry, such as bone healing, the immune response, inflammation, and nociception [1, 2]. In fact, a healthy, robust circadian rhythm is crucial for overall well-being, and circadian clock dysregulation or misalignment has been considered a risk factor for many diseases such as cancer, diabetes, and neurodegeneration [3-10]. Oral and craniofacial health is closely intertwined with general well-being, and dentistry is a multidimensional discipline that comprises a wide range of pathologies, surgical and non-surgical interventions, and rehabilitation [11].

Chronotherapy is a therapeutic modality tailored to the body's circadian rhythms to improve medical intervention outcomes. Mainly, two therapeutic approaches of chronotherapy have been adapted. First, regulation of the sleep/wake cycle rhythms using light therapy or sleep medications thereby modifying sleep patterns to re-synchronized disrupted circadian rhythms (e.g., affective disorders) [12]. Second, rescheduling interventions or restricting drug administration (Drug Chronotherapy) to the time of day in which it would be more effective with fewer adverse effects. For example, nighttime administration of anti-hypertensive drugs showed promising results in better-controlling blood pressure [13, 14].

Chronotherapy in dentistry is a relatively new subject; thus, we have found no review articles in which existing evidence in chronotherapy regarding oral and craniofacial health outcomes or its applications in dental practice has been synthesized. The overall aim of my thesis is to investigate

chronotherapy in oral health and post-operative recovery. The first objective of my PhD was to systematically map the research carried out regarding chronotherapy in dentistry. The second objective of my PhD was to evaluate an innovative pain management regimen utilizing chronotherapy in humans. This objective builds on our preclinical study evaluating Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) chronotherapy postoperative recovery in an animal fracture model, showing that restricting the intake of NSAIDs to the active phase in mice achieves faster recovery and superior pain control [15].

I address these objectives with three manuscripts; First, I conducted a scoping review to systematically map the research carried out regarding chronotherapy in dentistry and identify any existing gaps in knowledge. The second objective was addressed with two manuscripts with two different study designs. We proposed two randomized clinical trials (RCT) to evaluate the effect of NSAIDs chronotherapy on pain management and healing using an impacted third molar extraction model.

2 Chapter 2: Literature review

2.1 Introduction

This literature review will provide an overview of the circadian rhythm and its implications for overall health and well-being. Then, I will describe its role in oral health, bone health, and post-operative pain. Finally, I will give an overview of chronotherapy and its use of analgesics.

2.2 The Circadian Clock: An Overview

The circadian system at the molecular level comprises several positive and negative autoregulatory transcription-translation feedback loops (TTFLs) that control the expression of clock-controlled genes (CCGs). These feedback loops, in turn, cause clock genes to oscillate for approximately 24 hours, consequently influencing behavioral and physiological processes [16]. The core elements involved in the circadian rhythms are circadian locomotor output cycles kaput (CLOCK), neuronal PAS domain protein (NPAS2), brain and muscle ARNT-like protein 1 (BMAL1), PERIOD (PER1, PER2 and PER3), CRYPTOCHROME (CRY1 and CRY2), retinoic acid-related orphan nuclear receptors (ROR) α/β/γ and REV-ERB α/β [17].

Positive regulators such as CLOCK/NPAS2 and BMAL1 undergo heterodimerization to form a complex that initiates the transcription of several CCGs, namely PER 1/2/3 and CRY 1/2, in addition to ROR α/β/γ and REV-ERB α/β by binding to an E-Box enhancer region on their promoters [18]. After PER and CRY translate and accumulate in the nucleus, reaching a certain level, they inhibit BMAL1::CLOCK transcriptional activity, possibly by Casein Kinase 1 δ (CK1δ), causing phosphorylation and dissociation from the E-Box [19]. In other words, the PER::CRY complex suppresses its own transcription, thereby serving as a core negative feedback

loop. Alongside this core negative feedback loop, there are additional loops that are activated by positive and negative regulators of BMAL1 transcription, namely ROR α/β/γ and REV-ERB α/β. ROR α/β/γ promote BMAL1 expression while REV-ERB α/β act as suppressors as both compete to interact with the ROR response (RORE) motif on the BMAL1 promoter [20].

Furthermore, in gene expression cycling, post-translational control and modifications and protein turnover are crucial in regulating and resetting clock components to generate a period of approximately 24 hours [16, 21]. In fact, kinases regulate the phosphorylation of BMAL1, CLOCK, CRY, and PER following their synthesis in the cytoplasm and, therefore, play an important role in clock protein stability and transcription [22]. For instance, adenosine monophosphate-activated protein kinase (AMPK) phosphorylates and destabilizes CRY1/2, whereas PER1/2 is phosphorylated by either CK1δ/ε or CK2, resulting in their ubiquitination and degradation [23, 24]. CK2 also phosphorates BMAL1, causing its nuclear accumulation [25]. Moreover, CLOCK phosphoration by AKT causes its nucleolar accumulation, while its phosphorylation by glycogen synthase kinase-3 beta (GSK-3β) initiates CLOCK degradation [26, 27]. The abovementioned dynamics of transcriptional activation and/or suppression, in addition to the protein subcellular localization and degradation, provoke oscillations in CCGs' circadian expression, thereby influencing behavioral and physiological processes.

2.3 Clock-Modulating Small Molecules

The role of clock-modulating small molecules and their therapeutic potential have recently gained increasing attention in clock-related diseases. These small molecules can act directly (activation/inhibition) on core clock components or with key regulatory mechanisms (non-core

clock components) that significantly alter or control the circadian clock [28-30]. Clock-modulating small molecules provide a novel strategy that directly manipulates the circadian clock to improve medical outcomes intrinsic to disease etiology [31]. A famous example would be Jetlag, which is essentially a phase misalignment and thus can be targeted by clock-modulating small molecules with phase-resetting properties [31].

As mentioned above, small modulating molecules can directly or indirectly act on several core and non-core clock components. KL001, a carbazole derivative, was identified as the first clock-modulating small molecule directly targeting CRY [32, 33]. KL001 inhibits ubiquitin-dependent degradation (stabilization) of CRY, lengthens the circadian period, and dampens BMAL1 amplitude [32, 33]. This is interesting because, through CRY protein stabilization and negative regulations of genes encoding gluconeogenesis enzymes, KL001 opens a unique approach to treating diabetes [34]. Another small molecule is KS15, a derivative of 2-ethoxypropanoic acid, which blocks the interaction between BMAL1 and CRY proteins, subsequently enhancing the E-Box mediated transcription activity [33, 35]. Also, KS15 has shown that it can increase PER2 expression, thereby improving chemosensitivity and tumor-suppressor capacity in human breast cancer cells [36]. Further, CK1(a circadian non-core protein) regulates PER1/2, BMAL1, and CRY proteins through phosphorylation, and CK1 inhibitors (e.g., PF-670462) have been found to lengthen the circadian period [37, 38].

Moreover, LYC-55716 (Cintirogon) is an oral, small molecule RORγ agonist that increases T cell immune activity and decreases immune suppression in tumors [39]. Using a phase 1 open-label clinical trial, Mahalingam et al. investigated cintirogon's safety, tolerability, and antitumor activity

in 32 adults with advanced cancers [39]. They concluded that Cintirogon was safe and tolerable and selected a dose of 450mg BID for their phase 2 study assessing its clinical activity [39].

2.4 Circadian Clock and Oral Health

A healthy, robust circadian rhythm is crucial for overall well-being, and circadian clock dysregulation or misalignment has been considered a risk factor for many health disorders [3-6]. The circadian clock controls the devolvement and hemostasis of oral and craniofacial structures [40]. In fact, there are several circadian clock genes involved in maintaining oral health, and these clock genes are present in various craniofacial tissues such as the oral mucosa, epithelium, teeth (enamel, dentine, and pulp), periodontal ligaments (PDL) and salivary glands [40, 41]. For instance, fibroblast cells of human gingiva and periodontal ligaments expressed circadian core clock genes such as CLOCK, BMAL1, CRY1/2, and PER 1/2/3, suggesting their potential role in periodontal health and disease [42]. Further, clock genes and proteins (BMAL1, CLOCK, and PER1/2) were expressed in all major salivary glands (serous acini and duct cells), and aquaporin-5 (Aqp5), a water channel gene, was identified as a key target of clock genes that regulates salivary fluid secretions [43]. Such key findings combined with preliminary results that circadian clock alterations (PER2 and BMAL1 knockout in mice) could be linked to reduced saliva flow in Sjögren syndrome patients, subsequently providing a novel avenue of treatment modalities in salivary gland disorders [44]. Further, Zheng et al. observed that CLOCK, BMAL1, and PER1/2 were expressed in dental tissues with different intensities (up/down-regulated) in various embryonic stages of tooth development and PDL cells [45]. Interestingly, when comparing healthy and carious pulp tissues, PER2/3 and other genes were downregulated in the carious pulp tissues [46].

Moreover, 16 clock genes and their diurnal oscillations have been detected in healthy oral mucosa [47]. Indeed, Bjarnason et al. reported that PER1, CRY1, and BMAL1 have a circadian expression where PER1 peaks in the early morning, CRY1 peaks in the late afternoon, and BMAL1 peaks at night [48]. These rhythmic expressions also coincide with cell-cycle phases. That is, PER1 expression aligns with p53 (G1-phase marker), and BMAL1 aligns with cyclin β1(M-phase marker), thus suggesting their important role in oncogenesis [48].

Circadian clock genes have been linked to cancer development, prognosis, and therapy. Specifically to head and neck carcinomas (including oral carcinomas), many clock genes have been associated with cell proliferation and apoptosis regulation, cell cycle progression, and tumor volume and suppression, namely BMAL1, PER1/2/3, DEC1/2, CRY1/2, NPAS2, TIMELESS (TIM), RORα, and REV-ERBα [49-53]. For instance, PER1/2/3, CRY1/2, CKIϵ, and BMAL1 were significantly downregulated in head and neck cancer tissues [49]. In addition, PTEN is a tumor suppressor gene, and its oxidation-driven function loss causes BMAL1 to be upregulated in oral cancer [54]. Lastly, PER3, CRY2, and BMAL1 decreased expression in head and neck carcinomas was associated with more advanced larger tumor size, deeper tumor invasion, and worst survival rates [49].

2.5 Circadian Clock and Bone Health

In humans, healthy bone undergoes constant remodeling throughout life by a strictly regulated and balanced process of bone resorption by osteoclasts and bone formation by osteoblasts [55]. This bone turnover process is responsible for repairing microscopic cracks and healing fractures [55]. Circadian clock genes such as PER, CRY, and BMAL have been identified in bone tissue and

cells, and they play significant roles in bone formation and resorption [56-58]. For example, studies found generally that PER and CRY are negative regulators of bone while BMAL is a positive regulator that ultimately influences bone volume regulation [56, 59, 60]. Specifically, PER2 can control cell division cycle, and the loss of PER2 would increase bone proliferation [61]. Also, BMAL1 regulates mesenchymal stem cell differentiation to mature osteoblast in mice, and overexpression of BMAL1 has been reported to increase osteoblast differentiation [62, 63]. In addition, CRY2 was found to influence osteoclastic activity, while PER2 controls osteoblastic activity [64].

2.6 Circadian Clock and Post-Operative Pain

The inflammatory response following surgical trauma stimulates the wound repair process and is a major protective part of the innate immune reaction [65]. Wound repair cascade features overlapping phases, namely, homeostasis, inflammation, proliferation, and tissue remodeling [66]. Pain, a cardinal inflammation sign, is associated with the acute inflammatory reaction in which proinflammatory peptides substance P, calcitonin gene-related peptide, and neurokinin A are released from C-fibers in damaged tissue [65]. Depending on injury severity and patient biological factors, the duration of this acute inflammatory response varies between 24 hours and two weeks [65].

Furthermore, the pain system in humans exhibits circadian variation in perception and responsiveness throughout the day [67-70]. In a meta-analysis, Hagenauer et al. 2017 reported that pain sensitivity in healthy humans peaked at the end of the day and during the night [71]. Various clinical and experimental studies reported that disrupted circadian rhythm directly influences pain thresholds [72]. For instance, breakthrough pain in cancer patients peaks in the late morning/ early

afternoon [73, 74]. Both fibromyalgia and rheumatoid arthritis patients experience the highest pain levels in the early mornings [75, 76]. Also, toothache pain was reported to peak during the night [77]. Finally, a recent systematic review reported that post-operative pain peaks in the morning and troughs in the evening [78].

2.7 Chronotherapy

Chronotherapy, an emerging field in health research, aims at improving the therapeutic efficacy and pharmacokinetic profile and decreasing the side effects of drugs by selecting optimal dosing time to a specific circadian time window [79-82]. The theoretical foundation of this approach lies in the fact that the circadian clock regulates the expression of several therapeutic targets and proteins that play a key role in drug metabolism, thus influencing efficacy and pharmacokinetics [83]. Indeed, studies have demonstrated that more than 80% of FDA-approved drugs might show a diurnal variation of their mRNA expression levels and corresponding functions [84, 85]. Also, research reports that various top-selling medications in the US would achieve superior results if taken at a particular time of the day [86, 87]. The pharmacological effects of any medication is determined by absorption, distribution, metabolism, and elimination (ADME) processes [88]. These ADME processes can be regulated by another set of circadian clock-mediated processes, such as phase I and II drug-metabolizing enzymes and phase III detoxification transporter protein expression, gastric pH, gastrointestinal motility, and xenobiotic efflux by the blood-brain barrier [81].

2.8 Chronotherapy of Analgesics

The duration and nature of pain determine the choice of analgesics [69]. Acetaminophen, NSAIDs, and opioids are indicated for acute and nociceptive pain [69]. Since the 1980's, multiple animal and human studies have shown circadian variations in pharmacokinetics and effects of NSAIDs, and these studies have reported that NSAIDs' bioavailability peaks in the morning [69, 89, 90]. Further, NSAIDs seem to have maximum absorption and effectiveness when taken during the day [90-92]. A recent study from our lab using a tibia fracture surgical model in mice reported that NSAIDs are most effective in managing post-operative pain when they are given during the active phase [93].

3 Chapter 3: Rationale

Despite the increasing relevance of chronotherapy for health outcomes, little is known about this approach to oral health outcomes and dental treatments. This is surprising considering that oral and craniofacial health is viewed as a gateway and reflection of one's general health, well-being, and quality of life. Poor oral and craniofacial health has been constantly associated, directly and indirectly, with systemic diseases, malnutrition, and mental health disorders, consequently resulting in compromised quality of life [94-98]. Oral and craniofacial health is closely intertwined with general well-being, and dentistry is a multidimensional discipline that comprises a wide range of pathologies, surgical and non-surgical interventions, and rehabilitations. Therefore, it is unsurprising that a healthy and synchronized circadian rhythm is essential in maintaining proper oral and craniofacial health, and it is reasonable to assume that chronotherapy in such a field is promising. The overarching goal of my PhD project is to investigate the effect of chronotherapy on oral health and its application in dental practice.

Chronotherapy in dentistry is a relatively new subject, and I was unable to locate any review articles that synthesized existing evidence in chronotherapy regarding oral and craniofacial health outcomes or its applications in dental practice. Thus, the first aim of my PhD was to conduct a scoping review to systematically map the research carried out regarding chronotherapy in dentistry and identify any existing gaps in knowledge. Scoping reviews are an ideal methodology to examine emerging evidence as they provide a big picture of the available literature. Such a synthesis of the available literature is of importance because peripheral circadian clocks are present in the oral mucosa, enamel, dentin, dental pulp, and periodontal and bone tissues, thus influencing oral epithelium and saliva gland homeostasis and tooth development [42, 44, 48, 99-103]. Moreover,

dysregulated circadian clock components in shift workers have been linked to oral cancer development [50-53] and significant oral health problems [104].

Second, my PhD aims to evaluate an innovative pain management regimen utilizing chronotherapy in humans. This objective builds on our preclinical study evaluating NSAIDs chronotherapy on post-operative recovery in an animal fracture model showing that restricting the intake of NSAIDs to the active phase in mice achieves faster recovery and superior pain control [15]. We proposed two RCTs to test the effect of NSAIDs chronotherapy on pain management and healing using an impacted third molar extraction model. The choice of a surgical model of third molar (wisdom teeth) extraction to evaluate NSAIDs chronotherapy was based on the evidence that: (i) the model is recognized by the US Food and Drug Administration (FDA) and has a higher assay sensitivity as opposed to joint replacement and soft tissue surgical models [105, 106]; (ii) the model has been validated to investigate systematic inflammation using a protein secreted in the blood plasma in response to systematic inflammation called C-reactive protein (CRP) [107]; (iii) third molars surgical extraction under local anesthesia is one of the most common oral surgical procedures [108]. Indeed, the are over 5 million patients in the US extract their wisdom teeth at an estimated cost of $3 billion each year [109]. (iv) bone removal, a common procedure in these surgeries, is associated with pain and discomfort, and may strongly impact patients' QoL (i.e., a patient's ability to participate in regular life activities) [110-112]. Lastly, (v) NSAIDs are safe, effective and routinely prescribed to reduce pain and edema following oral and maxillofacial surgery [113, 114].

4 Chapter 4: Aims and Objectives

The overall aim of my PhD project is to investigate chronotherapy in oral health and its application in dental practice and to evaluate an innovative pain management regimen utilizing chronotherapy in a third molar extraction model. First, I evaluate the evidence regarding chronotherapy in dentistry and identify any existing gaps in knowledge. Then, I tested whether the administration time of NSAIDs affects pain perception and healing following impacted third molar extraction featuring two RCT study designs.

4.1 Specific objectives

The specific objectives of my PhD work are:

1- To conduct a scoping review to systematically map the research carried out regarding chronotherapy in dentistry, and to identify any existing gaps in knowledge. My research question was: What is known from the literature about the application of chronotherapy in dentistry?

2- To conduct a parallel RCT to evaluate NSAIDs chronotherapy on pain management and healing following impacted third molar extraction. The research question was: among patients undergoing surgical 3rd molar extraction, to what extent will the administration of NSAIDs during the morning and early afternoon result in better postoperative recovery compared to a routine standard care regimen?

3- To conduct a cross-over RCT to evaluate NSAIDs chronotherapy on pain management and healing following impacted 3rd molar extraction. The research question was: among patients with bilateral impacted mandibular third molars indicated for extraction, to what

extent will the administration of NSAIDs during the morning and early afternoon result in better postoperative recovery than the routine standard care regimen?

5 Chapter 5: Methodology

The three manuscripts of this thesis include a scoping review and two manuscripts presenting the results of NSAIDs chronotherapy RCTs (Parallel vs Cross over). This chapter presents an overview of the techniques, study designs, and analyses I performed in this thesis for each manuscript. The method section of each of the manuscripts further describes the methodology.

5.1 Methods of Manuscript I: Scoping Review

I used a scoping review methodology following the Systematic Reviews and Meta-Analyses (PRISMA) extension for Scoping Reviews guidelines [115], and the protocol was registered in the Figshare database [116].

5.2 Scoping review

One of the most commonly used definitions of scoping reviews is the one described by Arkesy and O'Malley in 2005: "Scoping studies aim to map rapidly the key concepts underpinning a research area and the main sources and types of evidence available, and can be undertaken as standalone projects in their own right, especially where an area is complex or has not been reviewed comprehensively before" [117]. This definition was revised by Daudt et al 2013: "Scoping studies aim to map the literature on a particular topic or research area and provide an opportunity to identify key concepts, gaps in the research; and types and sources of evidence to inform practice, policymaking, and research" and further suggested to augment scoping reviews with some form of quality assessment of included studies [118]. Scoping reviews' definition was further refined by Colquhoun et al 2014: "A scoping review or scoping study is a form of knowledge synthesis that addresses an exploratory research question aimed at mapping key

concepts, types of evidence, and gaps in research related to a defined area or field by systematically searching, selecting, and synthesizing existing knowledge" [119]. Further, scoping reviews are beneficial in informing research policy and practice [117, 120, 121]. Also, scoping reviews can determine the feasibility of systematic reviews, identify gaps in knowledge, and summarize existing literature [117]. In contrast to scoping reviews, systematic reviews answer a narrowly defined specific question [122, 123]. Further, scoping reviews require critical reinterpretation of the literature, whereas literature reviews do not [124].

The paragraph above highlights why I have chosen scoping review framework to answer my research question. First, oral health and dentistry are multidisciplinary fields and evidence thus far supporting chronotherapy is scarce. Second, chronotherapy is a broad field that includes altering sleep/wake cycle rhythms using light therapy or sleep medications and rescheduling interventions or restricting drug administration to a certain time of the day. Finally, it will give us an overview of various applications of chronotherapy in a multidisciplinary field such as dentistry. Therefore, a scoping review is the best choice as it helps to formulate more specific questions than a systematic review can effectively address.

After I conducted a quick literature review to formulate the research question and determine the tools/frameworks that could be used to answer the review question, I followed Arksey and O'Malley 2005 five stages to complete this scoping review as described below.

1- Identifying the research question

This scoping review aimed to systematically map the evidence underpinning chronotherapy in dentistry and identify knowledge gaps. We formulated the following research question: "What is known from the literature about the application of chronotherapy in dentistry?"

2- Identifying relevant studies

A medical librarian trained in knowledge synthesis techniques (MM) conducted a systematic scoping search of the literature to identify candidate articles [125]. A strategy for Ovid Medline was constructed using Medical Subject Headings (MeSH) and keywords, and Boolean operators (AND/OR) to combine the concepts of dentistry/oral health, circadian rhythms, and chronotherapy. This was then translated to Embase (Ovid), CINAHL and Scopus (Supplemental Table 1). Searches were carried out on June 30th, 2022. The language of articles was limited to English to match our team's expertise, while no restrictions were placed on publication year.

3- Study Selection

A PRISMA extension for Scoping Reviews [126] diagram outlining the article selection process is shown in Figure 1. Duplicates were removed using EndNote X9 citation management software (Philadelphia, PA, US). Candidate articles were then independently screened for title/abstract, and then full text, by two independent reviewers (MA, MT); any disagreements were resolved by a third author (WA). Agreement between the reviewers was substantial with a Cohen's kappa score of 0.662.

Furthermore, only original animal and human studies investigating the chronotherapeutic use of drugs or interventions in dentistry were included. As to our exclusion criteria, references were

excluded if they contained no abstract, were unrelated to dentistry, did not include a timed intervention in their methodology, or did not mention chronotherapy.

4&5- Data Charting and Data Synthesis

Two co-authors extracted and charted (MA and MT) the data from the included articles using Excel. Data were then summarized including each study's title, authors, publication year, study design, population, sample size, comparator groups, exposure, outcome, and results. Next, MA synthesized the collected data by combining both quantitative and qualitative approaches, which is suitable for amalgamating heterogenous studies and data [127]. Risk of bias assessment tools are used to evaluate and critically appraise the quality of included studies. For included studies, two blinded co-authors (KT and WC) independently performed quality critical appraisal using the Joanna Briggs Institute (JBI) assessment tools for human studies [128] and the SYstematic Review Center for Laboratory animal Experimentation's (SYRCLE) risk of bias assessment tool for animal studies [129]. These tools were used to assess included studies' internal validity and risk of bias. JBI critical appraisal tools were used in this study because JBI's checklists have the widest applicable range for human studies (e.g., RCTs, non-RCTs, Cohorts and Case reports). In addition, the SYRCLE checklist is considered the most recommended tool to use for animal studies [130]. These quality appraisal checklists and tools contains multiple questions assessing various domains evaluating internal validity and risk of bias. "Yes" indicates low risk of bias, "No indicates high risk of bias and "Unclear" means unclear risk of bias (Check lists are included in Appendix I).

5.3 Methods of Manuscripts II & III: Randomized Controlled Trial

Randomized controlled trials are prospective clinical studies that properly evaluate the effectiveness of new interventions or treatments by randomly assigning participants into an experimental group or a control group [131]. Randomization in such studies reduces bias by balancing participants' demographics (both recorded and unrecorded) between experimental groups [131]. RCTs provide a robust tool to investigate the cause-effect relationship between outcome and intervention. For these reasons, RCTs are considered a gold standard for clinical research [132]. Control groups in RCTs could be placebo-controlled or standard care. There are four main limitations of RCTs: 1- Time-consuming and high cost; 2- Limited generalizability (i.e., external validity), as participants might not represent the population; 3- Loss to follow up; 4- Ethical issues, as participants are exposed to certain risks of experimental interventions or missing out potential benefits when assigned to control groups.

Overall, there are four stages of clinical trials. Phase 1 typically focuses on safety and proper dosage of the new treatment. Next, Phase II clinical trials investigate efficacy and side effects. Phase III clinical trials then compare the new treatment to the standard of care in the targeted condition. Finally, Phase IV are conducted after treatments are approved and to follow up patients regarding long-term benefits and risks.

Furthermore, there are various study designs for RCTs, such as Parallel, Cross-over, and Factorial. Parallel groups RCT design is when each group of patients is exposed to only one of the study interventions. Typically, parallel-group designs are simple, straightforward, and easy to include in meta-analyses. In RCTs with parallel group designs, increasing the sample size increases the

accuracy of the results due to better representation of the population [133]. However, larger sample sizes also increase financial costs. The decision to increase the sample size to bolster the external validity so the findings can be extrapolated to large populations is delicate. A careful evaluation of the potential advantages in terms of external validity against the economic feasibility of conducting a more extensive trial is needed. Next, Cross-over designs, in which each patient receives both interventions but in a different order, ensure that all patients are exposed to all levels of independent variables; patients can serve as their own control, thus eliminating confounding subtle differences between comparison groups. Further, a smaller sample size is required as the within-patient variation is less than the between-patient variation [134]. One more advantage of cross-over design is that it partially eliminates the ethical issue of placebo as patients receive both treatments [135]. However, a washout period is paramount to avoid the carryover effect from the first intervention, and cross-over designs have a higher chance of dropout as they tend to take longer [135, 136]. The obvious limitations of such designs are the complexity of inclusion criteria for two independent treatments and the complexity of protocol and statistical analysis [135]. Another way of looking at RCT study designs is the level used for randomization. For example, instead of individual participants being randomly allocated for treatment, Cluster RCTs allocate intervention randomly to collections or clusters of patients [137].

Moreover, while some trials are designed to prove superiority, that is, Drug A (experimental) is better than Drug B (Gold Standard), non-inferiority trials demonstrate that a new treatment is not worse than an active control treatment by a predefined margin. They typically compare the new treatment to an established standard treatment, to show that the new treatment is at least as effective as the standard treatment, within a specified margin of difference. Finally, Factorial RCTs achieve

two clinical trials for the price of one. In other words, Factorial clinical trials answer two research questions with two different treatments on the same study population under the condition of interventions being independent. In my thesis, I chose Parallel and Cross-over designs for my second and third objectives.

5.3.1 Protocol registrations and ethical approvals

The RCTs in Manuscripts II and III were reported based on the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement [138], and their protocols were registered on ClinicalTrials.gov. ClinicalTrials.gov is a website and online database with the purpose of providing information on clinical research studies to the public, researchers, and healthcare professionals. In addition, the studies were approved by the Research Ethics Committee on Humans- Institutional Review Board (IRB) at Jordan University for Science and Technology (protocol number 393/2017) and the Research Ethics Committee at the San Carlos Clinical Hospital of Madrid, Spain (Trial registration code CEIC 19/216-R_M_BNI.) and the Agencia Española del Medicamento y Productos Sanitarios (AEMPS: Spanish Agency of Drugs and Sanitary Products, EUDRACT number 2019–000,736), respectively.

5.3.2 RCT Study Design

This thesis used two different RCT study designs. In Manuscript II, the clinical trial was a single-center, parallel-group, randomized, double-blind, placebo-controlled study. In Manuscript III, the clinical trial was a randomized, double-blinded, placebo-controlled, cross-over design study. Cross-over design ensures that all patients are exposed to all levels of independent variables, and patients can serve as their own control, thus eliminating confounding subtle differences between

comparison groups. Also, a smaller sample size will be required as the within-patient variation is less than the between-patient variation [134].

5.3.3 Blinding and Randomization

5.3.3.1 Manuscript II

In the interest of minimizing bias, both patients and surgeons were blinded to treatment group allocation. After undergoing clinical evaluation, patients who met the eligibility criteria were randomized into two experimental groups. One of the investigators not involved in the recruitment or treatment performed generated a list of randomization sequences using permuted-block randomization via the website www.randomization.com. Sequentially numbered, opaque, sealed envelopes were used to shield the investigators responsible for the treatment and recruitment of the new patients into the trial from knowing upcoming assignments. Each envelope contained the group assignment for one patient. The same investigator also distributed the ibuprofen and the placebo in the appropriate sequence. Other than these tasks, this person had no contact with the study's participants and no involvement in data collection or analysis. All the records and the results of the tests were identified with codes known only by a research assistant who did not participate in the study.

5.3.3.2 Manuscript III

To reduce bias for this pilot study, both patients and surgeons were blinded to treatment group allocation. Second-year surgical resident performed all the surgical procedures (FPG). Both third molar side and medication (3 times ibuprofen or two times ibuprofen + placebo) randomization were performed by the main investigator (JTGD) using a coin flip. Other than providing

medications, the investigator did not have any contact with the study's participants or involvement in data collection.

5.3.4 Data Management

5.3.4.1 Manuscript II

All data recorded from the RCT in Jordan was paper-based and was entered into the FileMaker database. Next, all missing data were checked and re-entered after consulting the original paper-based records. Once the database was completed on FileMaker, it was exported as a CSV file, which then was imported into R Studio for data cleaning, visualization, and analysis. Data cleaning was performed to look for missing data, errors in data entry, unreasonable values, etc. Any issue thereafter was resolved before statistical analysis.

5.3.4.2 Manuscript III

All data collected from the RCT in Spain was paper-based and was entered into Microsoft Excel Sheet. Next, all missing data were checked and re-entered after consulting the original paper-based records. Once the database was completed, it was imported into R Studio for data cleaning, visualization, and analysis. Data cleaning was performed to look for missing data, errors in data entry, unreasonable values, etc. Any issue thereafter was resolved before statistical analysis.

5.3.5 Study Outcomes

The target outcomes were pain relief and healing indicators. Below I describe in detail how the outcomes were measured.

5.3.5.1 Primary Outcome Measures

5.3.5.1.1 Pain measurements

Our primary outcome on Manuscripts II and III was pain severity. Pain intensity was recorded using a segmented numeric version of the Visual Analog Scale (VAS), which is valid, reliable, and appropriate for use in clinical trials and practice [139]. We used the Arabic version of VAS scale because this study was conducted in Jordan.

5.3.5.2 Secondary outcome measures

We used proxy bone-healing indicators, including facial swelling and mouth opening. We used Laskin's method to measure facial swelling. While facial swelling three-dimensional volume change is challenging to evaluate, the proposed method is commonly used among researchers and has shown some reliability pertaining to its easy clinical application [140]. To assess postoperative swelling, we measured: a) Horizontal distance to the symphysis (DHS); the distance in millimeters from the bottom edge of the earlobe to the midpoint of the symphysis Hirota, b) Horizontal distance to the corner (DHC); the distance in millimeters from the bottom edge of the earlobe to the external angle of the mouth, and c) Vertical distance (DV); the distance in millimeters from the palpebral outboard angle to the gonial angle. Trismus, an inability to open the mouth, may be a consequence of the surgery due to inflammatory processes involving the muscles. We evaluated this condition by measuring the distance between the incisal edges of the upper and lower central incisors with a ruler when the mouth is fully open. Trismus was considered present if the patient has limited jaw opening less than or equal to a 10-mm interincisal distance [141]. The presence of erythema, muscle tenderness, tempers mandibular symptoms and dry socket were checked and recorded during the follow-up visit.

5.3.5.2.1 *Additional Explanatory Measures*

We used the expression levels of CRP, measuring the inflammatory level as one of our explanatory variables in Manuscript II, with wound healing. CRP, synthesized by the liver, is a protein secreted in the blood plasma in response to systematic inflammation. This secretion may be as a response to trauma or surgery such as third molar surgical extraction. CRP level less than 10 mg/L is considered to be normal, and the peak of the inflammatory response is at 72-96 hours postoperatively with a progressive reduction after one week [142]. Previous studies showed that CRP is an excellent objective indicator for the inflammatory clinical response following third molar surgical extraction, and its elevated level was associated with clinical signs of acute inflammation such as facial swelling and limited mouth opening [107, 142].

5.3.6 Data analysis

5.3.6.1 Descriptive statistics

Descriptive statistics were performed to observe the distribution of socio-demographic characteristics and clinical parameters between the intervention (chronotherapy) and control groups. For continuous variables such as age, surgery duration, baseline measurements of pain, facial swelling and trismus, and no. of local anesthesia capsules used, median and interquartile range (IQR) was obtained. As for categorical variables such as sex, oral hygiene, smoking habits, education level, annual income, tooth impacted, type of impaction and surgical complication, percentages were calculated.

5.3.6.2 Statistical Analysis

5.3.6.2.1 Linear mixed-effects models

We used Linear mixed-effects models (LMMs) to evaluate the effect of NSAID chronotherapy on pain perception after third molar extraction. LMMs are adopted to consider correlated observations within subjects. These models are extensions of simple linear models, allowing both fixed and random effects [143]. They are typically used in repeated measures that come from a hierarchical structure. In addition, LMM allows for the understanding of not only the overall trend in the data but also the individual variations among the subjects that we are studying. For example, we observed the pain levels of our participants, which might differ from each other over, for several days. These differences can affect the overall pain over the week (fixed effect). This model also considers individual differences (each person is unique) the random effect. As for missing VAS pain scores, we compared both models with missing values and imputed values, and there was no difference in results due to a small number of missing values.

- The following equation was used to fit our model in R Studio:

LMM= VAS Pain Scores ~ Day +Time + Assignment + Day: Assignment + Time:Assignment + Day: Time + (1|ID).

- Day: we collected pain measures for four days, so it was either Day 1, Day 2, Day 3, or Day 4.
- Time: each day, we collected three pain scores which are 12 points for the whole study period.
- Assignment: Intervention or Control.

5.3.6.2.2 Kruskal-Wallis H test

The Kruskal-Wallis H test was used to compare pre- and post-measurement testing differences between experimental groups, and the. Kruskal-Wallis H test is a nonparametric rank-based test to determine significant differences of comparison between two on more groups [144].

5.3.6.2.3 Wilcoxon signed rank

For the quantitative variables, Wilcoxon signed rank test was conducted. Wilcoxon signed rank test is a nonparametric test for matched-pair data analysis [145] .

5.3.6.2.4 McNemar's chi-squared test

McNemar's chi-squared test was performed for qualitative variables. McNemar test is a paired binary data analysis that compares discordant pairs of responses in favour of each intervention [146].

A preface to the manuscript I

This chapter summarize the available literature on the applications of chronotherapy in dentistry.

6 Chapter 6: Manuscript I

Title: Chronotherapy in Dentistry: A Scoping Review

Mohammad Abusamak[1], Mohammad Al-Tamimi[1], Haider Al-Waeli[1], Kawkab Tahboub[1], Wenji Cai[1], Martin Morris[1], Faleh Tamimi[1], and Belinda Nicolau[1]

1. Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada
2. Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia, Canada
3. Schulich Library of Physical Sciences, Life Sciences and Engineering, McGill University, Montreal, Quebec, Canada
4. College of Dental Medicine, QU Health, Qatar University, Doha, Qatar

Corresponding author:

Mohammad Abusamak, Faculty of Dental Medicine and Oral Health Sciences, McGill University, 2001 McGill College Avenue, Montreal, QC, Canada

Email: mohammad.abusamak@mail.mcgill.ca

Published in the Chronobiology International Journal on April 13th, 2023.

https://doi.org/10.1080/07420528.2023.2200495

Abstract

The circadian clock modulates almost all vital aspects of our physiology and metabolism, including processes relevant to dentistry, such as healing, inflammation and nociception. Chronotherapy is an emerging field aiming to improve therapeutic efficacy and decrease adverse effects on health outcomes. This scoping review aimed to systematically map the evidence underpinning chronotherapy in dentistry and to identify gaps in knowledge. We conducted a systematic scoping search using four databases (Medline, Scopus, CINAHL and Embase). We identified 3908 target articles screened by two blinded reviewers, and only original animal and human studies investigating the chronotherapeutic use of drugs or interventions in dentistry were included. Of the 24 studies included, 19 were human studies and five were animal studies. Chrono-radiotherapy and chrono-chemotherapy reduced treatment side effects and improved therapeutic response, leading to higher survival rates in cancer patients. Animal studies reported that tooth movement and periodontal tissue response to orthodontic forces follow a diurnal rhythm that might influence bone metabolism. Profound and prolonged local anesthesia could be achieved when injected in the evening. Although the overall quality of the included studies was low, chronotherapy applications in dentistry seem to have favourable outcomes, especially in head and neck cancer treatments.

Keywords: Drug chronotherapy; circadian rhythm; oral health; chronobiology; dentistry

6.1 Introduction

In humans, circadian rhythms (~ 24h) control all major organ systems, thus orchestrating nearly all vital aspects of our physiology and metabolism, including processes very important to dentistry, such as bone healing, the immune response, inflammation, and nociception [1, 2]. These biological rhythms coordinate our brain with other tissues to perform distinct, likely inharmonious, functions pertinent to the day and night cycle, which in turn, strongly influence overall physical and mental health [147]. Indeed, a healthy robust circadian rhythm is crucial for overall well-being, and circadian clock dysregulation or misalignment has been considered a risk factor for many diseases such as cancer, diabetes, and neurodegeneration [3-10]. The circadian rhythm is regulated by molecular pathways comprising several positive and negative transcription-translation feedback loops (TTFLs) that control the expression of clock-controlled genes (CCGs). These feedback loops, in turn, cause clock genes to oscillate for approximately 24h cycles, consequently influencing behavioral and physiological processes [16]. The core circadian elements involved in the circadian rhythms are circadian locomotor output cycles kaput (CLOCK), neuronal PAS domain protein (NPAS2), brain and muscle ARNT-like protein 1 (BMAL1), PERIOD (PER1, PER2 and PER3), CRYPTOCHROME (CRY1 and CRY2), retinoic acid-related orphan nuclear receptors (ROR) α/β/γ and REV-ERB α/β [17].

The circadian clock controls the devolvement and hemostasis of oral and craniofacial structures [40, 148]. Several circadian clock genes present in craniofacial tissues (e.g., oral mucosa, epithelium, teeth (enamel, dentine, and pulp), periodontal ligaments and salivary glands) are involved in maintaining oral health [40, 41, 44, 148]. For instance, fibroblast cells of human gingiva and periodontal ligaments express circadian core clock genes such as CLOCK, BMAL1,

CRY1/2, and PER 1/2/3, suggesting their potential role in periodontal health and disease [42]. Further, clock genes and proteins (BMAL1, CLOCK, and PER1/2) are expressed in all major salivary glands (serous acini and duct cells), and were found to regulate salivary fluid secretions through the action of the water channel gene aquaporin-5 [43]. Moreover, circadian clock alterations (PER2 and BMAL1 knockout in mice) may be linked to reduced saliva flow in Sjögren syndrome patients. Such key findings provide novel avenues for treating salivary gland disorders [44]. Clock genes (i.e., CLOCK, BMAL1 and PER1/2) are also expressed in dental tissues (up/down-regulated) at various embryonic stages of tooth development [45] and could be downregulated in pathological dental conditions such as deep caries [46].

Moreover, clock genes and their diurnal oscillations have been detected in the healthy oral mucosa [47]. For example, PER1, CRY1 and BMAL1 have different circadian expression peaks in the early morning, late afternoon and at night, respectively [48]. These rhythmic expressions also coincide with cell-cycle phases, that is, PER1 expression aligns with p53 (G1-phase marker), and BMAL1 aligns with cyclin β1(M-phase marker), thereby suggesting their important role in oncogenesis [48].

Chronotherapy is a therapeutic modality tailored to the body's circadian rhythms to improve medical interventions outcome [88]. Mainly, two therapeutic approaches of chronotherapy have been adapted [149]. First, altering sleep/wake cycle rhythms using light therapy or sleep medications thereby modifying sleep patterns to re-synchronized disrupted circadian rhythms (e.g., affective disorders) [12]. Second, rescheduling interventions or restricting drug administration (Drug Chronotherapy) to the time of day that would be more effective with fewer

adverse effects[150]. For example, the nighttime administration of anti-hypertensive drugs showed promising results in better controlling blood pressure [13, 14]. Also, chronotherapy of commonly prescribed medications for allergic rhinitis and bronchial asthma, at a certain time of the day, yielded better therapeutic outcomes and/or fewer adverse effects [151]. Likewise, chronotherapy shows a potential avenue to improve cancer survival by reducing toxicities of anti-cancer treatments (chemotherapy and radiotherapy), which therapeutic choices and doses are often limited due to the side effects severity [152-154]. In addition, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) chronotherapy may to improve recovery from bone fracture in mice [155]. Finally, it has been recently hypothesized that chronotherapy could be utilized to better manage severe COVID-19 complications, wherein anti-inflammatory drugs are administered in the afternoon targeting detrimental cytokines only [156].

Based on the abovementioned observations, this scoping review aimed to systematically map the existing literature in chronotherapy in dentistry and identify gaps in knowledge. This type of review identifies, maps, collates and summarizes the literature to assist researchers in recognizing fundamental ideas, theories, evidence sources and gaps in knowledge in the field of interest [117, 157]. In contrast to systematic reviews, scoping reviews incorporate the "Big Picture" in the underlying literature rather than answering a narrowly defined specific question [127]. In our case, we choose a scoping review methodology because oral health and dentistry are multidisciplinary fields and evidence thus far supporting chronotherapy is scarce. Also, chronotherapy is a broad field that includes altering sleep/wake cycle rhythms using light therapy or sleep medications and rescheduling interventions or restricting drug administration to a certain time of the day. Therefore, a scoping review is the best choice as it will help to formulate more specific questions than a

systematic review can effectively address. Additionally, it will give us an overview of various applications of chronotherapy in a multidisciplinary field such as dentistry.

6.2 Methods

This scoping review was reported according to the Systematic Reviews and Meta-Analyses (PRISMA) extension for Scoping Reviews guidelines [126].

6.2.1 Protocol Registration

This review protocol was registered [116] in the figshare database (https://doi.org/10.6084/m9.figshare.20431683.v1, accessed on August 5th, 2022).

6.2.2 Identifying the research question

This scoping review aimed to systematically map the evidence underpinning chronotherapy in dentistry and identify knowledge gaps. We formulated the following research question: "What is known from the literature about the application of chronotherapy in dentistry?"

6.2.3 Identifying relevant studies

A medical librarian trained in knowledge synthesis techniques (MM) conducted a systematic scoping search of the literature to identify candidate articles [125]. A strategy for Ovid Medline was constructed using Medical Subject Headings (MeSH) and keywords, and Boolean operators (AND/OR) to combine the concepts of dentistry/oral health, circadian rhythms, and chronotherapy. This was then translated to Embase (Ovid), CINAHL and Scopus (Supplemental

Table 1). Searches were carried out on June 30th, 2022. The language of articles was limited to English to match our team's expertise, while no restrictions were placed on publication year.

6.2.4 Study Selection

A PRISMA extension for Scoping Reviews [126] diagram outlining the article selection process is shown in Figure 1. Duplicates were removed using EndNote X9 citation management software (Philadelphia, PA, US). Candidate articles were then independently screened for title/abstract, and then full text, by two independent reviewers (MA, MT); any disagreements were resolved by a third author (WA). Agreement between the reviewers was substantial with a Cohen's kappa score of 0.662.

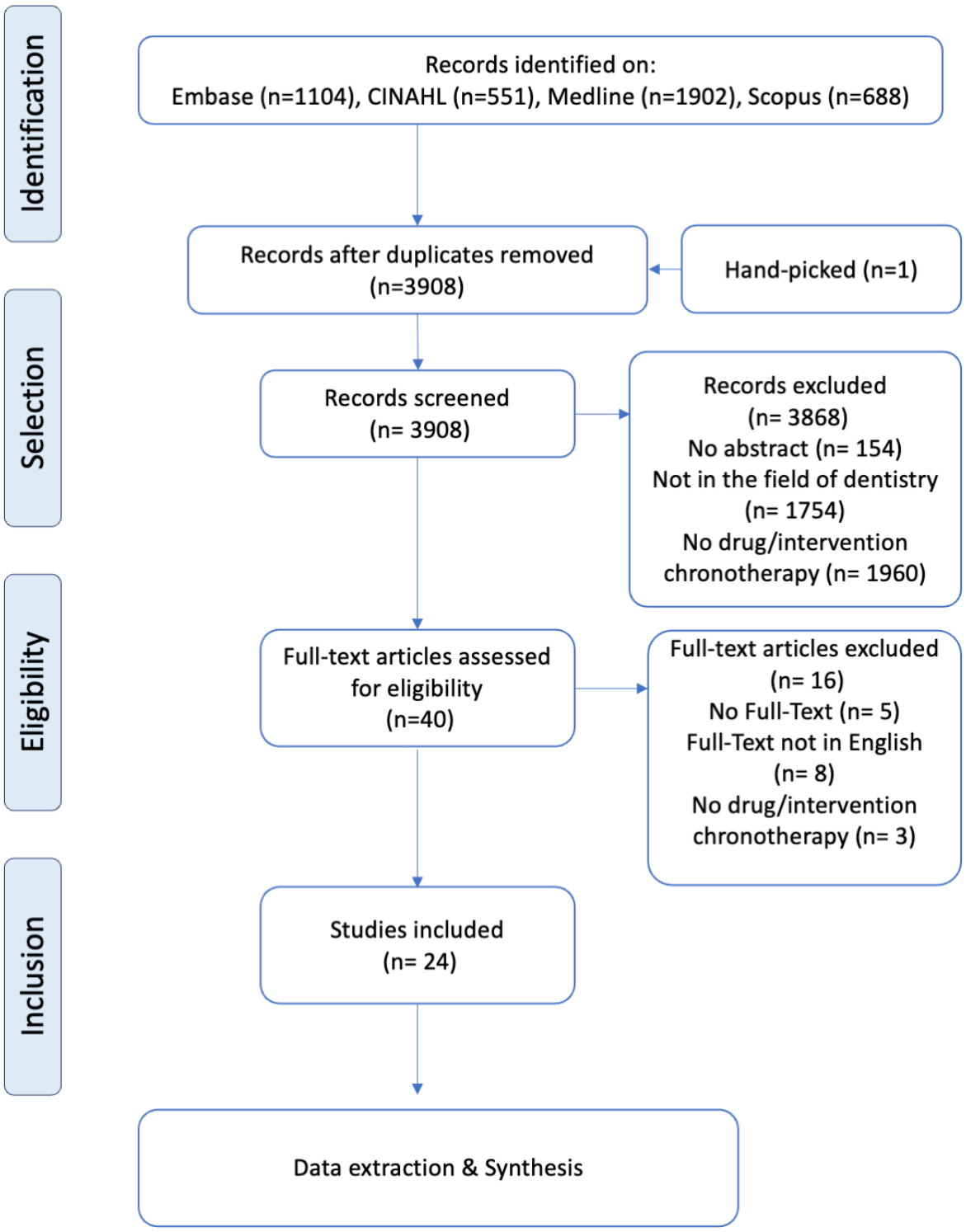


Figure 1: PRISMA flowchart of selected studies.

Furthermore, only original animal and human studies investigating the chronotherapeutic use of drugs or interventions in dentistry were included. As to our exclusion criteria, references were excluded if they contained no abstract, were unrelated to dentistry, did not include a timed intervention in their methodology, or did not mention chronotherapy.

6.2.5 Data Charting and Data Synthesis

Two co-authors extracted and charted (MA and MT) the data from the included articles using Excel. Data were then summarized including each study's title, authors, publication year, study design, population, sample size, comparator groups, exposure, outcome, and results. Next, MA synthesized the collected data by combining both quantitative and qualitative approaches, which is suitable for amalgamating heterogenous studies and data [127]. For included studies, two blinded co-authors (KT and WC) independently performed quality critical appraisal using the Joanna Briggs Institute (JBI) assessment tools for human studies [128] and the SYstematic Review Center for Laboratory animal Experimentation's (SYRCLE) a risk of bias assessment tool for animal studies [129]. These tools were used to assess included studies' internal validity and risk of bias. JBI critical appraisal tools were used in this study because JBI's checklists have the widest applicable range for human studies (e.g., Randomized Controlled Trial (RCTs), non-RCTs, Cohorts and Case reports). In addition, the SYRCLE checklist is considered the most recommended tool to use for animal studies [130].

6.3 Results

Following the screening process, 24 articles were retained after applying the inclusion and exclusion criteria. Of the 24 studies assessed and outlined in Table 1, 19 (79%) and 5 (21%) were human and animal studies, respectively. Among those human studies, 11 were clinical trials, 7 were retrospective cohorts and one case report. The number of articles published per year was not evenly distributed. Five general areas of research emerged: Studies on chemotherapy and radiotherapy treatments in head and neck carcinomas (n=15); studies on orthodontic forces and tooth movement (n=3); studies on local anesthesia (n=2); studies on prosthodontics and oral medicine (n=2); and studies on post-operative pain management and surgery (n=2). All chemotherapy and radiotherapy included studies (n=15) were within the scope of dentistry. Furthermore, a detailed overview of the included studies is outlined in Supplemental Table 2. Table 2 presents studies investigating drug chronotherapy and reported targeted pathways.

Table 1: Overview of included studies in the scoping review

First Author/Year	Design	Population (n)	Outcome Measured
Chrono-chemotherapy			
Yang et al. 2013 [158]	Animal	Mice (n=75)	Therapeutic response & adverse effects
Chen et al. 2013 [159]	Retrospective	Humans (n=49)	Therapeutic response & adverse effects
Zhang et al. 2021 [160]	Retrospective	Humans (n=150)	Therapeutic response & adverse effects
Verma et al. 2014 [161]	RCT	Humans (n=60)	Therapeutic response & adverse effects
Zhang et al. 2018 [162]	RCT	Humans (n=148)	Therapeutic response & adverse effects
Lin et al. 2013 [163]	RCT	Humans (n=125)	Therapeutic response & adverse effects
Tsuchiya et al.2018 [164]	Crossover	Humans (n=9)	Adverse effects only
Chrono-radiotherapy			
Zhang et al. 2013 [165]	Animal	Mice (n=366)	Therapeutic response only
Gu et al. 2020 [166]	Retrospective	Humans (n=190)	Adverse effects only
Kuriakose et al. 2016 [167]	Retrospective	Humans (n=142)	Adverse effects only
Brolese et al. 2021 [168]	Retrospective	Humans (n=617)	Adverse effects only
Elicin et al. 2021 [169]	Retrospective	Humans (n=655)	Therapeutic response only
Goyal et al. 2009 [170]	RCT	Humans (n=212)	Therapeutic response & adverse effects
Bjarnason et al. 2009 [171]	RCT	Humans (n=216)	Therapeutic response & adverse effects
Elzahi et al. 2020 [172]	Non-RCT	Humans (n=160)	Adverse effects only
Orthodontic Forces &Tooth Movement			
Igarashi et al. 1998 [173]	Animal	Rats (n=30)	Tooth movement, bone formation & resorption
Miyoshi et al. 2001 [174]	Animal	Rats (n=100)	Tooth movement, bone formation & resorption
Yamada et al. 2002 [175]	Animal	Rats (n=37)	Condylar growth inhibition & chondrocytes differentiation & proliferation
Local Anesthesia			
Lemmer and Wiemers 1989 [176]	Non-RCT	Humans (n=83)	Numbness duration
Pöllmann 1982 [177]	Non-RCT	Humans (n=67)	Numbness duration & pain onset after surgery
Prosthodontics & Oral Medicine			
Latta Jr 1992 [178]	Non-RCT	Humans (n=30)	Centric relation positional changes edentulous
Waghmare & Puthenveetil 2021 [179]	Case Report	Humans (n=1)	Complete remission
Pain Management & Surgery			
Tamimi et al. 2022 [180]	RCT	Humans (n=70)	Postoperative pain & healing
Restrepo et al 2020 [181]	Retrospective	Humans (n=187)	Postoperative complications (immediate & late)

Table 2: Included studies investigating drug chronotherapy.

Drugs	Half-life	Reported Targeted Pathways in Included Studies
Taxanes		
Paclitaxel	8.83 ± 4.10h [182]	Not reported.
Docetaxel	12h [183]	Circadian rhythm of DNA synthesis and cell proliferation were detected in the bone marrow and gastrointestinal mucosa and these biological rhythms could influence docetaxel adverse effects [164].
Platinum-based antitumor		
Cisplatin	51 ± 22 min (i.v./6 hr) [184]	Glutathione (GSH) is an antioxidant that prevents cell damage and toxicities caused by Cisplatin are associated with the circadian variation of GSH that peaks at 16:00h [185].
Carboplatin	118 +/- 15 mins [186]	Not reported.
Antimetabolites		
5- Fu	12.9 ± 7.3 min [187]	Adapting 5- Fu administration to the daily rhythm of the principal enzyme of degrading 5- Fu, Dihydropyrimidine dehydrogenase (DPD), thus producing less toxicity [188].
Anti-Inflammatory Drugs		
Ibuprofen	2h [189]	Postoperative pain, swelling, and CRP serum levels peak during the day (Pro-inflammatory phase) [180]
Prednisolone	2.1- 3.5h [190]	Prednisolone administration with peak levels of bodily cortisol (i.e., 08:00h) showed minimum cumulative cortisol suppression and maximum effects on the action of lymphocytes [191].
Local Anesthesia		
Mepivacaine	1.6h [192]	Circadian variations in distribution, metabolism, elimination processes, nerve cell membrane permeability and access to ion channels. Efflux of K and its cell concertation lowest at 15:00h [193].
Articaine	0.5h [192]	

The risk of bias assessment of the included studies is summarized in Supplemental Table 3. Overall, RCTs did not properly conceal groups allocation, and neither patients nor investigators were blinded to treatment assignment, intervention, and outcome assessment. On the other hand, non-RCTs displayed low risk of bias. While the included cohort studies generally exhibited low risk of bias, about 70% did not identify confounding variables nor adjusted for them in their analysis. Lastly, randomization and sample size calculation were mainly lacking in animal studies attributing to the high risk of bias. Years of publication varied extremely across research areas. For instance, all studies on head and neck cancer were fairly recent and published between 2009 and 2021. In contrast, studies in prosthodontics treatments and local anesthesia were published between 1982 and 1992. Also, orthodontic forces and tooth movement animal studies were conducted between 1998 and 2002. Figure 2 illustrates the best time for different interventions reported in the included studies.

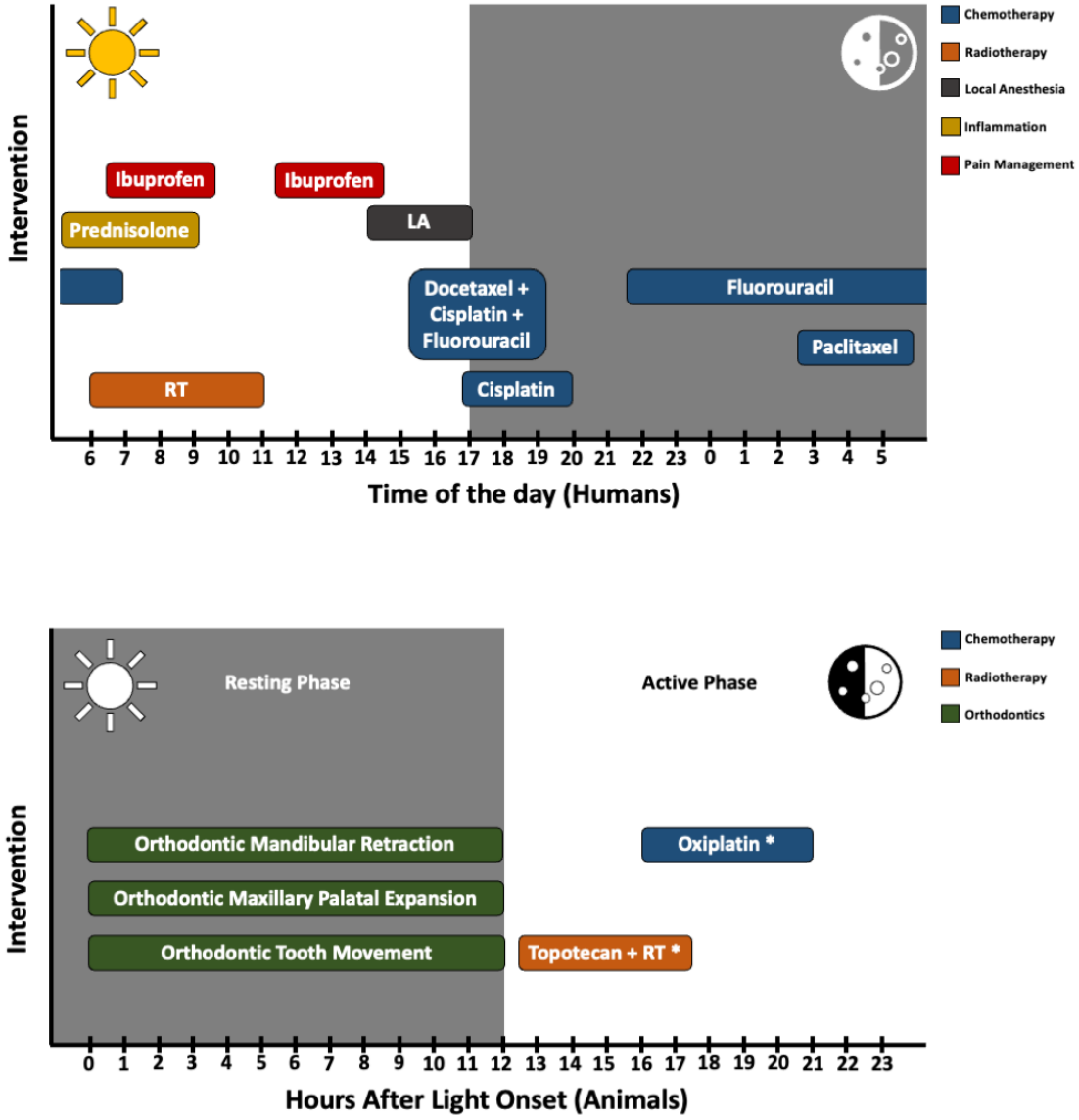


Figure 2: Best time for different interventions reported in the included studies. Abbreviations: RT: Radiotherapy; LA: Local Anesthesia for restorative and surgical procedures; *: Studies conducted on mice. Ibuprofen and Prednisolone were prescribed for pain management after third molar extraction surgery and treating Oral Pemphigus, respectively.

6.3.1 Head and Neck Cancer

6.3.1.1 Chrono-chemotherapy

Of the 24 articles included in this review, four RCTs, two retrospective cohort studies and one animal trial assessed chrono-chemotherapy.

6.3.1.1.1 Animal Studies

In the animal study, Yang et al. 2013 investigated how dosing time influences the efficacy and side effects of oxaliplatin (L-OHP) on oral squamous cell carcinoma in mice. L-OHP injected at 16 and 22 Hours After Light Onset (HALO) significantly increased survival rate and greatly reduced adverse effects compared to L-OHP injected at 4 and 10 HALO [158].

6.3.1.1.2 Human Studies

In the retrospective cohort studies, Chen et al. 2013 compared chronomodulated chemotherapy (i.e., Paclitaxel (03:00h – 05:00h) and Carboplatin (16:00h – 20:00h) on Day 1, while 5-Fluorouracil (5-Fu) on Day 1 to 5 at 22:00h – 07:00h) and conventional (Control) chemotherapy started between 09:00h – 11:00h and finished before 17:30h. They reported that the chronomodulated chemotherapy group had a significantly lower overall incidence of adverse effect with higher tumor response rate and longer patients' survival [159]. Zhang et al. 2021 investigated the efficacy and safety of induction chemotherapy combined with chrono-chemotherapy (i.e., Cisplatin 10:00h – 22:00h with peak delivery of at 16:00h) or conventional chemotherapy (i.e., intravenous instilling of Cisplatin 10:00h to 22:00h) in locally advanced nasopharyngeal carcinoma. Their work showed that chrono-chemotherapy could decrease incidence rates and severity of adverse reactions and improve treatment-induced immunosuppression [160].

Regarding the RCTs, two RCTs investigated chronomodulated Cisplatin administration. Verma et al. 2014 compared Cisplatin efficacy and toxicity when prescribed at 06:00h or 18:00h, followed by radical external bead radiotherapy in locally advanced head and neck carcinomas. By contrast, Zhang et al. 2018 compared Cisplatin chronomodulated infusion (10:00h – 22:00h with peak delivery at 16:00h) to flat intermittent Cisplatin infusion (10:00h – 14:00h), combined with intensity-modulated radiotherapy in locoregionally advanced nasopharyngeal carcinoma patients. They assessed adverse effects, immune function impairment, and therapeutic efficacy. While both studies concluded that Cisplatin chronotherapy received in the evening significantly reduced adverse effects and was better tolerated, there was no significant impact on cancer control or survival [161, 162].

The other two RCTs evaluated Cisplatin chronotherapy combined with other chemotherapeutic medications. For example, Lin et al. 2013 examined the therapeutic and toxic effects of Cisplatin and 5-Fu chronomodulated infusion (Cisplatin 10:00h – 22:00h with peak delivery of at 16:00h, and 5-Fu 22:00h – 10:00h with a peak delivery at 04:00h) as opposed to the same 12-hour time-period (for both medications) but with flat intermittent constant infusion rate, followed by radical radiotherapy in advance nasopharyngeal carcinoma patients. Patients prescribed Cisplatin and 5-Fu chronotherapy experienced significantly less stomatitis, but with similar therapeutic and toxic effects to the flat infusion [163]. Finally, a cross-over RCT design tested a chemotherapeutic regimen trifecta (Docetaxel, Cisplatin, and 5-Fu) in two periods (10:30h vs 18:30h) in oral squamous cell carcinoma patients[164]. The author observed a reduction in nausea (66.7% vs 22.2% $p < 0.05$) and other adverse effects (e.g., vomiting [33.3% vs 22.2%, $p > 0.05$] and

neutropenia [22.2% vs 11.1%, p>0.05]) in the evening-dosing [164]. Because this RCT had a cross-over design, the therapeutic response could not be assessed.

6.3.1.2 Chrono-radiotherapy

6.3.1.2.1 Animal Studies

In the animal study, Zhang et al. 2013 evaluated the chronomodulated effect of topotecan (TPT), a radiosensitizing agent, in a human nasopharyngeal carcinoma mouse model. Their work revealed that TPT's radiosensitivity effect is time-dependent, and TPT combined with radiotherapy achieved a superior therapeutic effect on delaying tumor regrowth when administered at 15 HALO (active period) [165].

6.3.1.2.2 Human Studies

Two retrospective studies included in this review investigated whether radiotherapy timing could influence oral mucositis severity in managing head and neck cancer. Indeed, both studies demonstrated that radiation-induced oral mucositis was significantly lower when patients were exposed to radiotherapy in the morning [166, 167]. In contrast, Brolese et al. 2021 reported that the seasonality, not daytime (morning/evening), was a predictor for radiation-induced oral mucositis severity. Patients who underwent radiotherapy between September and March (winter) suffered an increased incident of acute toxicities [168]. Furthermore, while investigating the same cohort, Elicin et al. 2021 found that patients who underwent radiotherapy in the winter had superior loco-regional control and progression-free survival [169].

Moreover, three clinical trials (2 RCTs and 1 non-RCT) studied the effect of radiotherapy in the morning vs. afternoon on radiation-induced oral mucositis severity in head and neck carcinomas patients. Goyal et al. 2009 and Bjarnason et al. 2009 randomly assigned head and neck carcinomas patients to morning and afternoon groups (08:00h – 11:00h vs 15:00h – 18:00h & 08:00h – 10:00h vs 14:00h – 16:00h, respectively). Both studies stated that the morning radiotherapy group exhibited reduced oral mucositis severity grades [170, 171]. In the third clinical trial, although non-randomized, investigators were blinded to groups allocation (06:00h – 08:00h vs 13:00h – 15:00h) and participants were matched according to age, sex, and tumor site [172]. Nevertheless, similar results were reported regarding the association between radiotherapy time of the day and oral mucositis severity grades (i.e., the morning radiotherapy group had less severe form of mucositis) [172].

6.3.2 Orthodontic Forces and Tooth Movement

Two studies investigated orthodontic forces [173] (i.e., Maxillary expansion) and tooth movement [174] as a function of time, while a third study examined mandibular retractive forces during the resting period [175]. Findings from these three studies conducted on rats and in the same Japanese lab demonstrated that restricting orthodontic forces to a certain time of day would achieve better outcome [173-175]. For instance, maxillary expansion and tooth movement were faster in the light-period group (07:00h – 19:00h) with increased new bone formation on the tension side [173, 174]. On the other hand, orthodontic forces were far less effective during the dark-period (19:00h – 07:00h) [173, 174]. Although all-day force application achieved similar tooth movement to the light-period group, the light-period group had less extensive periodontal ligament hyalinization

[174]. Also, mandibular retractive forces were a more effective when applied during the light-period (08:00h-20:00h) [175].

6.3.3 Local Anesthesia

This review included two studies that investigating local anesthesia injected at different times of the day in humans. Lemmer and Wiemers 1989 used an electronic pulp tester to measure the stimulus threshold of anterior teeth. They quantified the total local anesthesia effect by time to reach peak effect, duration at peak effect and time to return to baseline threshold. On the other hand, Pöllmann 1982 measured numbness duration and pain onset after oral surgery. In addition to reporting circadian behavior of local anesthesia [176, 177], both studies showed the maximal drug effect was achieved when local anesthesia was injected at 14:00h and 17:00h [176]. Moreover, the longest duration of local anesthesia was achieved when injected at 15:00h, while the shortest duration was at night and early morning [177].

6.3.4 Prosthodontics and Oral Medicine

Latta Jr 1992 found that centric relation records for complete denture fabrication showed a circadian variation [178]. For instance, if centric relation records are taken in the morning, fabricated complete dentures thereafter would better fit the patient's mouth in the morning and vice versa. So, It was suggested that treating edentulous patients in the middle of the day would dilute such circadian changes [178].

In the included case report treating Oral Pemphigus Vulgaris [179], showed that a single dose of Prednisolone at 06:00h achieved complete remission of the oral lesion as opposed to conventional regimen (twice daily), which 50% reduction only.

6.3.5 Post-Operative Pain Management and Surgery

Tamimi et al. 2022 conducted an RCT evaluating the effect of chronotherapy of NSAIDs on post-operative recovery in a third molar extraction model. They concluded that restricting ibuprofen administration to daytime (morning and afternoon) might be as sufficient as conventional administration regimens (morning, afternoon and evening) in controlling post-operative pain after third molar extraction [180].

Restrepo et al. 2020 evaluated cleft lip and palate surgeries and their early and late complications. Among other parameters, they investigated the correlation between time of surgery (morning vs. afternoon) and post-operative complications. They reported no correlation between incident of complications and surgery time [181].

6.4 Discussion

Chronotherapy (interventional or drug) is a promising and unique therapeutic approach aimed at improving medical outcomes, ultimately leading to better overall health and well-being. Re-scheduling medical interventions and medications to a certain time of the day aligned with the body's biological rhythms are sought to provide a simple and cost-effective way to maximize treatment benefits while minimizing adverse effects. Oral health and dentistry are multidimensional fields, and healthcare professionals in such disciplines could greatly benefit from

applying chronotherapy to improve patients' quality of life. In this scoping review, we broadly mapped existing literature underpinning chronotherapy applications in dentistry and identified gaps in knowledge. We categorized the available literature into chronotherapy of head and neck cancer treatment, orthodontics, prosthodontics, oral medicine, local anesthesia, post-operative pain management, and surgery. Identifying knowledge gaps was attained by an in-depth review of included studies' quality (i.e., risk of bias assessment tools), number, population, and design in each research area.

Our findings showed that restricting chemotherapy and/or radiotherapy to specific time of the day might generally reduce treatment adverse events and relatively increases therapeutic response and survival rates in head and neck cancer patients [158-172]. However, these studies' findings and clinical implications should be interpreted with caution due to methodological limitations. First, specifically in RCTs, blinding patients and investigators to treatment allocation, intervention and outcome assessment was absent by design. In other words, study participants and investigators were aware to treatment assigned (e.g., morning vs. evening) that might prompt them to behave differently, thus rendering these studies at higher risk of bias and compromising their internal validity. While this limitation could be attributed the lack of feasibility to blindly conduct time-dependent interventions and using a placebo, the risk of bias should not be underestimated. Second, multiple medications (e.g., Paclitaxel, Carboplatin, 5-FU, Cisplatin and Docetaxel) are frequently prescribed when treating head and neck carcinomas with chemotherapy. This presents yet another challenge to developing a research protocol following a rigorous study design that minimize inconsistences and the risk of bias. In addition, almost all studies investigating chrono-chemotherapy had other cycles of non-time-stipulated induction chemotherapy and/or concurrent

radiotherapy. This overlap between chemotherapy and radiotherapy will influence, for instance, the severity of adverse events, thus diluting the treatment effect. Finally, current studies are insufficient to amend on-going guidelines. However, conducting systematic reviews and meta-analyses is feasible, which would provide the bases for optimized multicentre RCT designs.

Moreover, dental healthcare professionals could take advantage of prolonged and profound local anesthesia when injected in the afternoon [176, 177]. This is potentially useful for patients undergoing lengthy procedures such as root canal treatments and oral surgeries. This prolonged and profound anesthesia could be achieved because circadian rhythms influence the pharmacological sensitivity of many medications by regulating physiological functions and parameters essential to pharmacokinetics and pharmacodynamics [194, 195]. Similar to many U.S. Food and Drug Administration (FDA) approved drugs showing diurnal variation, local anesthesia efficacy and toxicity are time-dependent [83, 194]. However, chronotherapy of local anesthesia should be further investigated as the included studies were non-randomized, had a high risk of bias and were conducted more than 30 years ago. Further, NSAIDs chronotherapy (i.e., morning and afternoon administration only) was reported to be of potential therapeutic benefit for post-operative recovery after wisdom tooth extraction [180]. This finding was also in accordance with a recent study, published after our search was conducted, investigating the same outcomes but in a cross-over design [196]. However, these RCTs had relatively small sample size which could influence their reported findings. Accordingly, a multicentred RCTs with larger sample sizes would be essential in changing the current standard of care regimen (3-doses per day) for pain control after surgery. Figure 3 demonstrates proposed dental procedures that would benefit from the chronotherapy of NSAIDs and local anesthesia.

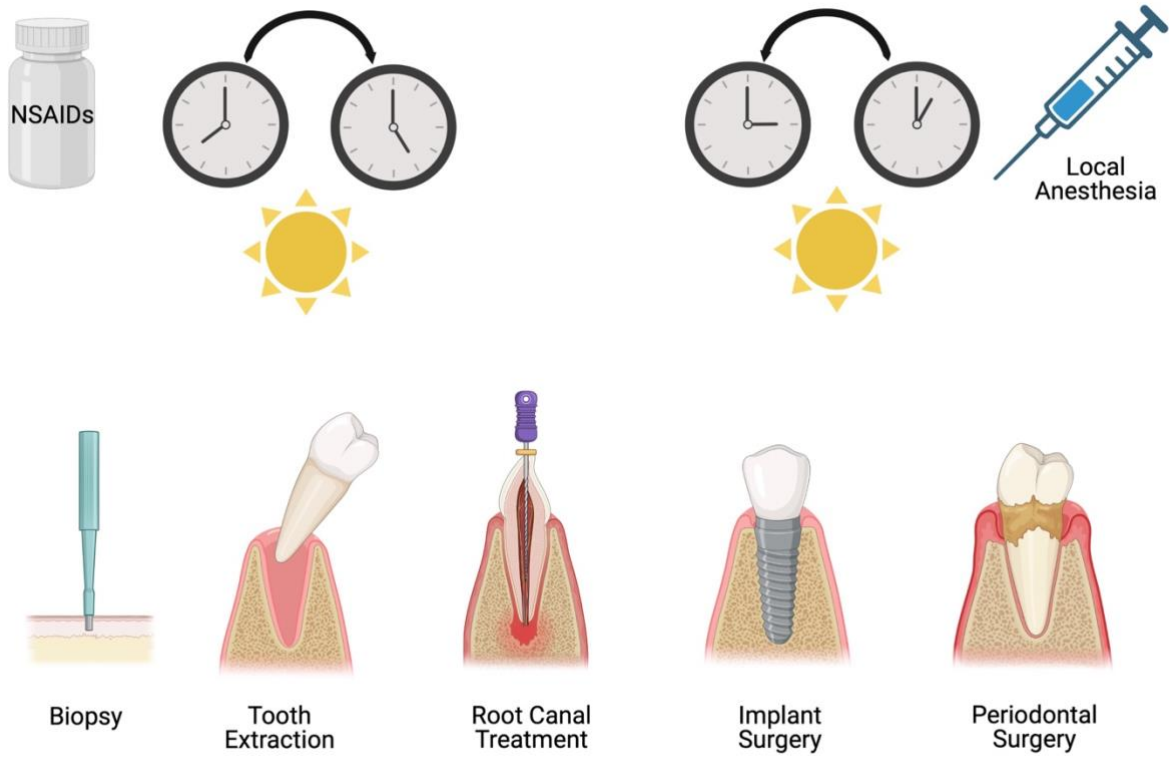


Figure 3: Proposed dental procedures that could benefit from chronotherapy of NSAIDS and Local Anesthesia. Clocks in the figure represent optimized dosing time for interventions. Figure was created with BioRender.Com.

In addition, our review showed that orthodontic forces applied in different times during the day would result in considerable variation in teeth movement in rats [173-175]. Various studies have shown that bone physiology, including metabolic markers and bone formation and resorption, exhibits diurnal variations [197, 198]. According to these circadian fluctuations in bone physiology, mechanically induced bone remoulding by orthodontic forces might be time-dependent and varies throughout the day and night. However, it is important to mention that rats are nocturnal animals, and the light-period is their resting phase. So, when translating these findings to humans, restricting orthodontic treatments to nighttime (intermittent force application for 12 hours) would be comparable to continuous all-day orthodontic treatment and would cause less damage to periodontal tissues (i.e., PDL hyalinization). Nevertheless, chronotherapy of orthodontic treatments should be further tested in humans to evaluate its feasibility and whether it could realistically reduce treatment duration.

Furthermore, there are several factors/modifiers have been reported to influence\alter one's circadian rhythm such as age, sex, chronotype, diet, sleeping habits, social interactions, exercise, disease status, smoking, and drugs [199, 200]. Most included human studies did not consider circadian rhythm modifiers in their analyses. Only one study looked in the chronotype of their studied population and three studies performed secondary subgroup analyses for circadian rhythm modifiers (Supplemental Table 4). Considering such modifiers is important; for example, Bjarnason et al. 2009 observed different trends in the chrono-radiotherapy effect between males and females, which could be attributed to gender-specific genes involved in various pathways including the cell cycle [201]. While a proper study design with randomization would eliminate the subtle confounding difference in recruited patients, considering circadian rhythm modifiers

would increase the internal validity and reliability. However, there are limitations in terms of feasibility and maintaining internal validity of the study (i.e., restricted inclusion/exclusion criteria) [200]. Nonetheless, prospective studies should consider incorporating circadian-based protocols in patient recruitment strategies, study design, and analyses.

The role of clock-modulating small molecules and their therapeutic potential have recently gained increasing attention in clock-related diseases. These small molecules can act directly (activation/inhibition) on core clock components or with key regulatory mechanisms (non-core clock components) that significantly alter or control the circadian clock [28-30]. Compared to traditional chronotherapy (i.e., explicit rescheduling of existing drugs to improve efficacy or/and reduce toxicity), clock-modulating small molecules provide a novel strategy that directly manipulates the circadian clock to improve medical outcomes intrinsic to disease etiology [31]. Jetlag is a famous example, which is essentially a phase misalignment and thus can be targeted by clock-modulating small molecules with phase-resitting properties [31]. Thus far, in a recently published thesis, SR9009 (i.e., clock-modulating small molecule) demonstrated chemotherapeutic benefits on squamous cell carcinoma cell lines [202]. However, more pre-clinical and clinical studies are needed to further our understanding of the potential therapeutic uses and benefits of clock-modulating small molecules.

6.5 Conclusions

All in all, chronotherapy applications in dentistry have shown favourable outcomes. Although evidence thus far suggest that chrono-chemotherapy and chrono-radiotherapy could be promising therapeutic regimens in head and neck cancer, standardized study protocols are needed. While

chronotherapy of orthodontic treatments in animal trials revealed promising results, human clinical trials are lacking. Prolonged and profound local anesthesia could be achieved when injected in the afternoon. However, chronotherapy of local anesthesia should be further investigated in an optimized study design. In addition, even though NSAIDs chronotherapy could be an effective and simple dosing regimen to better control post-operative pain after surgery, multicenter RCTs should be further conducted. Finally, centric records accuracy for complete denture fabrication showed time-dependency, while time of surgery showed no correlation with post-operative incidence of complications.

Acknowledgements:

The authors would like to thank Dr. Sohaib Al-Natsheh for his insightful discussions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The work was supported by the Canadian Institutes of Health Research [CIHR PJT-168875].

Author Contributions

Conceptualization: Mohammad Abusamak, Mohammad Al-Tamimi, Haider Al-Waeli, Faleh Tamimi and Belinda Nicolau; Methodology: Martin Morris and Mohammad Abusamak; Software: Mohammad Abusamak; Validation: Mohammad Abusamak and Mohammad Al-Tamimi; Formal analysis: Mohammad Abusamak and Mohammad Al-Tamimi; Investigation: Martin Morris and

Mohammad Abusamak; Resources: Mohammad Abusamak and Mohammad Al-Tamimi; Data Curation: Mohammad Abusamak, Mohammad Al-Tamimi, Kawkab Tahboub,

Wenji Cai and Haider Al-Waeli; Writing – original draft preparation: Mohammad Abusamak; Writing – review and editing: Mohammad Abusamak, Mohammad Al-Tamimi, Kawkab Tahboub, Wenji Cai, Martin Morris, Faleh Tamimi and Belinda Nicolau; Visualization: Mohammad Abusamak; Supervision: Faleh Tamimi and Belinda Nicolau; Project administration: Faleh Tamimi and Belinda Nicolau; Funding acquisition: Faleh Tamimi.

Supplemental data

https://doi.org/10.1080/07420528.2023.2200495

A preface to manuscript II

This manuscript investigates the effect of NSAIDs chronotherapy on pain management in impacted third molar extractions utilizing parallel groups design.

7 Chapter 7: Manuscript II

Title: NSAID chronotherapy after impacted third molar extraction: a randomized controlled trial

Zaid Tamimi[1], Mohammad Abusamak[2], Haider Al-Waeli[3], Mohammad Al-Tamimi[2], Rola Al Habashneh[1], Mohammad Ghanim[1], Mohammed Al-Nusair[4], Qiman Gao[2], Belinda Nicolau[2], and Faleh Tamimi[5]

1. Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan
2. Faculty of Dentistry, McGill University, Montreal, QC, Canada
3. Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia, Canada
4. Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
5. College of Dental Medicine, Qatar University, Doha, Qatar

Zaid Tamimi and Mohammad Abusamak are the first co-authors.

Corresponding author:

Zaid Tamimi, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan

Email: zztamimi@just.edu.jo

Published in the Oral and Maxillofacial Surgery Journal on January 22nd, 2022.

https://doi.org/10.1007/s10006-021-01029-8

Abstract

Objectives: Postoperative pain management impacts patients' quality of life and morbidity. Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are widely used for this following a 3-doses-per-day regime. However, pain and inflammation follow a circadian rhythm, and animal models assessing the scheduling of NSAID administration (e.g., chronotherapy) have shown that while their use during the active phase of the day enhances postoperative recovery, their administration during the resting phase could have detrimental effects. This observation has led us to hypothesize that night administration of NSAID might be unnecessary in post-surgical scenarios. Therefore, a randomized clinical trial was conducted to test this hypothesis in surgical third molar extractions.

Materials and methods: Seventy (18–35 years) healthy participants requiring surgical removal of impacted lower third molars were recruited and randomized into a double-blind placebo-controlled study. For three days postoperatively, the treatment group (n = 33) received ibuprofen (400 mg) at 8 AM, 1 PM, and a placebo at 8 PM, while the control group (n = 37) received ibuprofen (400 mg) at 8 AM, 1 PM, and 8 PM. Pain severity was assessed by visual analog scale (VAS) and healing indicators including facial swelling, mouth opening, and C-reactive protein blood levels were also measured.

Results: Pain VAS measures showed a circadian variation peaking at night. Also, no significant differences were observed between the two groups of the study in terms of postoperative pain scores (estimate: 0.50, 95% CI = [− 0.38, 1.39]) or any other healing indicator.

Conclusions: Postoperative pain follows a circadian rhythm. Moreover, night administration of ibuprofen might not provide any significant benefits in terms of pain management and control of

inflammation, and two doses during the day only could be sufficient for pain management after surgical interventions.

Knowledge transfer statement: Even though this study cannot rule out the possibility that a reduced regime is different than a standard regime, nocturnal doses of ibuprofen seem to have no clinical significance in the short term, and the results of this study provide evidence in favor of reducing ibuprofen administration from three doses to two doses only after third molar surgery.

Keywords: Pain management, Chronotherapy, NSAIDs, Molar, Third, Tooth extraction, Drug chronotherapy.

7.1 Introduction

The surgical extraction of third molars under local anesthesia is one of the most common oral surgical procedures. Each year more than 5 million patients in the USA are having their third molars (wisdom teeth) extracted at an annual cost of over $3 billion. Most of the third molars surgical extractions require bone removal [111], and lead to pain and discomfort due to the inflammatory process after surgery. Indeed, more than 11 million patient-days of "standard discomfort or disability" were reported due to surgical removal of third molars, with an average of 4.9 lost workdays per procedure [109, 112].

Currently, postoperative pain management is limited to acetaminophen, opioids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) [203, 204]. However, all these drugs have limitations. Acetaminophen is not effective in managing severe pain [204]. Opioids and NSAID are effective in pain management, but opioids can cause constipation and addiction [205, 206], and NSAIDs may delay bone healing [207, 208]. Dentists and maxillofacial surgeons all over the world prefer to prescribe NSAIDs after this type of surgery [114]. The mechanism of action of NSAIDs is the reversible inhibition of the enzyme cyclooxygenase (COX) that syntheses prostaglandins from arachidonic acid [208], which play a major role in inflammatory and nociceptive processes. Two isoforms of COX, namely, COX-1 and COX-2, have essential roles in the inflammatory process after bone surgery. While COX-1 is more involved in the integrity of the gastrointestinal and renal tract tissues, COX-2 is mainly involved in the inflammatory and healing process [209]. NSAIDs are either nonselective (inhibiting both COX-1 and COX-2) or selective (inhibiting COX-2 only). Ibuprofen, a peripherally acting analgesic that works on inhibition of both COX-1/COX-2, provides fast analgesic effect without increasing the risk of side effects [210]. It is routinely used

in the treatment of moderate to severe acute pain such as dental pain or postoperative discomfort [211-213].

Although systematic reviews have reported that higher doses and frequent use of NSAIDs are associated with better pain control after dental (e.g., third molar extraction) and non-dental surgeries [207, 214, 215], there is an increased risk of adverse effects. Moreover, despite these promising results, patients still report pain and other discomfort (e.g., swelling and trismus) especially during the first three days after third molar extraction surgeries [216]. These symptoms can affect the patients' activities of daily living and their quality of life [112, 216-218]. Importantly, animal[219], retrospective cohorts, and some clinical studies [220-224] suggest that NSAID affect bone healing outcomes. Therefore, there is a need to develop a better strategy to manage postoperative pain after third molar extraction. Treatments adapted to the inflammatory process may decrease its intensity and, thereby, pain. One promising avenue using this strategy is rooted in the body's biological functions that follow circadian rhythms.

In mammals, the circadian system is comprised of a central and peripheral clock that anticipate daily changes imposed by the environment [225, 226]. Bone healing and immune responses are modulated by such robust system [226]. For example, at the beginning of the active phase in the morning, macrophages activity, leukocytes recruitment, and proinflammatory mediators increases [225-228]. By contrary, angiogenesis factors and anti-inflammatory mediators peak during the resting phase at the evening [226] . Bone healing process is also modulated by the circadian rhythm oscillation, which occurs in bone tissue during growth, formation, and resorption [229-231]. In bone formation process, all bone cells, such as osteoblasts and osteoclasts express clock genes that regulate bone volume [60, 232]. Experimental studies in animals and humans have demonstrated

that disrupted circadian rhythm and sleep cycle may impair bone formation [55]. Further, the circadian clock during the active phase of the circadian rhythm influences pain response which could partially explain the daily changes in COX-1 and COX-2 activity in injured tissues [69, 233, 234]. These changes aligned to the circadian behavior of pharmacokinetics effects of NSAIDs, therefore, may provide clinical evidence that NSAIDs administration during the active phase may maximize their absorption and effectiveness [91, 92, 235-237].

The literature outlined above has led to the development of chronotherapy, which is the science of preventing or treating illness according to biological rhythms [238]. It involves the timing of pharmacological, medical, or surgical interventions to minimize side effects and increase treatment efficacy [91, 235, 236]. More than half of the available medications, including those over-counters, have their action regulated by circadian clocks making the timing of administration a promising treatment strategy [86]. Although this essential biological process has been largely ignored; many drugs may achieve maximum absorption and effectiveness when administered during the active phase [69]. The optimal time of the day for administration depends on the species, i.e., daytime for humans but night-time for several nocturnal animals (e.g., mice).

Results of our preclinical studies showed that administering NSAIDs at wake-up compared to bedtime cut in half the recovery time for pain and bone healing in mice, including mechanical and histomorphometric properties of the healing callus [239]. These findings support the role of the circadian rhythm in inflammation during bone healing and may have major implications for bone-related surgical interventions. Although chronotherapy has the potential to better control inflammation, decrease pain and recovery time, no clinical studies have yet investigated the

effectiveness of the timing of NSAIDs administration on postoperative healing outcomes in humans.

Based on our animal research we hypothesize that nocturnal administration of NSAID might be unnecessary in post surgical scenarios. Therefore, a randomized clinical trial was conducted to test this hypothesis in surgical third molar extractions. Ibuprofen is the most commonly used NSAID in oral surgery, and it is often prescribed at doses of 400 mg every 8 hours [141] . Ibuprofen serum concentration peaks 1 to 2 h after oral administration and is eliminated from our system to negligible levels within 9-10 hours [240]. Accordingly, our working hypothesis was that among patients undergoing third molar extraction surgery, strict diurnal administration of ibuprofen (two doses of 400 mg at 8 am and 1 pm) will result in better postoperative recovery than the routine standard of care of three doses of ibuprofen per day (400 mg at 8 am, 1 pm and 8 pm). In this experiment patients of both groups would have similar serum levels of ibuprofen during the day, but at night, only the control group would have clinically relevant levels, thus allowing us to better understand the effect of nocturnal doses of ibuprofen.

7.2 Materials and Methods

This study is reported based on the CONSORT 2010 statement [241]. A flow chart of this study is shown in (Figure 4).

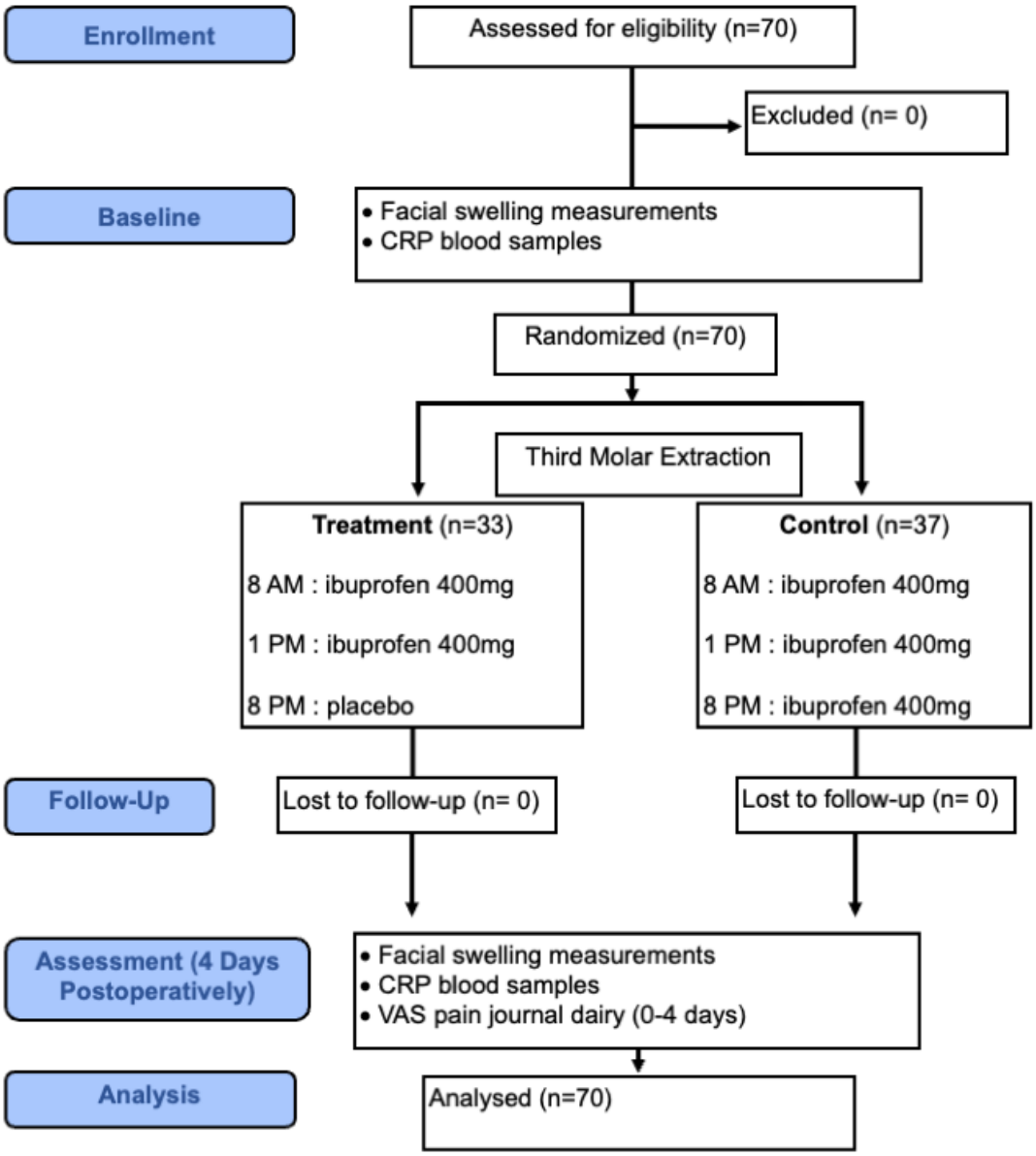


Figure 4: Study flowchart

7.2.1 Study design

This clinical trial was a single-centre, parallel-group, randomized, double-blind placebo-controlled study. Randomization took place at the individual level (1:1 ratio) and was carried out in the Dental Teaching Clinics at the Jordan University of Science and Technology in Irbid, Jordan. The study started in Aril 2018 and completed in February 2021. For three days after third molar extraction surgery, the treatment group (Chronotherapy) was instructed to take only two doses of ibuprofen (400 mg) (8 am and 1 pm) combined with a placebo at 8 pm, while the control group (Standard care regimen) was instructed to take three doses of ibuprofen (400 mg) at 8 am, 1pm and 8pm. The study was approved by the Research Ethics Committee on humans- Institutional Review Board (IRB) at Jordan University for Science and Technology and Jordanian Food and Drug Administration (JFDA). The trial was prospectively registered with the ClinicalTrials.gov, number NCT03789058.

7.2.2 Inclusion and exclusion criteria

Men and women aged between 18 and 35 years old were eligible if they (i) presented with a bony impacted lower third molar with the classification of Bell and Gregory Type B II, (ii) were healthy according to the American Society of Anesthesiologists (ASA) classification (subject should not have an active infection, trismus, hyperthermia, or swelling before surgery and must be able to maintain adequate oral hygiene), and (iii) had an adequate understanding of written and spoken English or Arabic to fill out questionnaires and informed consent forms. Other eligibility criteria include no previous history of systemic diseases (e.g., diabetes mellitus, hypertension, gastric ulcer), or severe/serious illness that requires frequent hospitalization. Also, those who were pregnant or breastfeeding, had impaired cognitive or motor function, and were taking anti-

inflammatory or analgesic drugs in the previous two weeks or are allergic to NSAIDs were not eligible for the study.

7.2.3 Recruitment and randomization

Participants who were interested in the study and fulfilled the inclusion criteria were asked to sign a consent form. In the interest of minimizing bias, both patients and surgeons were blinded to treatment group allocation. After undergoing clinical evaluation, patients who met the eligibility criteria were randomized into two experimental groups. One of the investigators not involved in the recruitment or treatment performed generated a list of randomization sequence using permuted-block randomization via the website www.randomization.com. Sequentially numbered, opaque, sealed envelopes were used to shield the investigators responsible for treatment and recruitment of the new patients into the trial responsible from knowing upcoming assignments. Each envelope contained the group assignment for one patient. The same investigator also distributed the ibuprofen and the placebo in the appropriate sequence. Other than these tasks, this person had no contact with the study's participants and no involvement in data collection or analysis. All the records and the results of the tests were identified with codes known only by a research assistant who did not participate in the study.

7.2.4 Intervention

All the surgeries were performed between 8 AM and 11 am by two surgeons (ZT and MG) using the same technique. For each surgery, a full thickness flap was lifted under local anesthesia (2% lidocaine with 1:100,000 epinephrine). A no. 701 surgical bur (drill) was used to perform osteotomy. The surgical site was inspected after extraction, and any sharp bone was filed to prevent discomfort. Copious irrigation was applied, followed by closure using 3/0 polyglactin 910 (Vicryl)

sutures. We recorded the tooth location (right or left), the volume of local anesthesia administered (the number of anesthetic tubes used), the time of the surgery (morning only), the time needed to perform the surgical procedure and the name of the surgeon.

Each participant received medications for three days postoperatively. The treatment group received two doses of ibuprofen before the afternoon, as well as a third dose consisting of a placebo (a sugar tablet of the same color and shape as the active drug tablet) at bedtime while the control group received the standard care, that is, three doses of ibuprofen 400 mg three times per day. The oral medications (ibuprofen and the placebo) were identical in appearance and were given to each patient by the investigator in a closed envelope labeled with the patients assigned study identification number.

7.2.5 Linear mixed-effects model estimating NSAID outcome measures

Our primary outcome was pain severity in the first three days after surgery. Patients recorded pain scores three times per day (8am, 1pm and 8pm) using a segmented numeric version of the Visual Analog Scale (VAS) [242], which has been previously validated in many RCTs [243, 244]. The VAS quantifies the amount of pain that a patient feels and ranges from 0 (no pain) to 10 (the worst pain imaginable). Patients were instructed to fill out a journal diary for 3 days after surgery and deliver the diary to the investigator during the follow-up visit. For ethical reasons, all participants received rescue medication (500mg acetaminophen), and they were instructed to take 2 tablets as needed, not to exceed 4 g/day. Patients were permitted take the painkiller whenever they felt considerable pain, per their judgment, and were instructed to wait at least 6 hours between doses

and write in the diary each time the medication was used. All participants were asked to record the types of food they ate and any side effects for three days after surgery.

In this study, we used proxy bone-healing indicators including facial swelling and mouth opening as our secondary outcome. A single investigator collected these measures at baseline (before the surgical procedure) and on day 4. To assess postoperative swelling, we measured: a) Horizontal distance to the symphysis (DHS); the distance in millimeters from the bottom edge of the earlobe to the midpoint of the symphysis Hirota, b) Horizontal distance to the corner (DHC); the distance in millimeters from the bottom edge of the earlobe to the external angle of the mouth, and c) Vertical distance (DV); the distance in millimeters from the palpebral outboard angle to the gonial angle. Trismus, an inability to open the mouth, may be a consequence of the surgery due to inflammatory processes involving the muscles. We evaluated this condition by measuring the distance between the incisal edges of the upper and lower central incisors with a ruler when the mouth is fully open. Trismus was considered present if the patient has limited jaw opening less than or equal to a 10-mm interincisal distance [141]. The presence of erythema, muscle tenderness, tempers mandibular symptoms and dry socket were checked and recorded during the follow-up visit.

We also evaluated the inflammatory condition associated with wound healing using the expression levels of Creative protein (CRP). A venous blood sample of 3 ml was drawn from the cubital fossa of each patient two times (once preoperatively and once three days postoperatively). The collected samples were analyzed in the JUST laboratory immediately, and the result was sent to the investigator by email using patient serial numbers. Blood CRP levels was tested one hour before the surgical procedure.

7.2.6 Statistical analysis

We required 30 participants in each arm to estimate a standardized difference of 0.25 in pain scores between the treatment and control arms with a power of 80% and Type I error rate of 5%. Therefore, a total of 73 participants were recruited and randomized in two arms, taking into consideration a 10% loss to follow-up. Our primary outcome measurement was postoperative pain relief (i.e., 3 days post-surgery). The effect of NSAIDs (ibuprofen) chronotherapy treatment on repeatedly measured postoperative VAS pain scores were examined using Linear mixed effects models (LMMs). These models are adopted to consider correlated observations within subjects. As per our secondary outcome measurements, trismus, facial swelling, and explanatory outcomes (serum concentration of inflammatory mediators), changes over time were tested by Kruskal-Wallis Rank Sum Test. All statistical analyses were conducted using the statistical program R 3.6.1 (R Foundation) and LMMS were performed using the "lme4" package in R [245].

7.3 Results

Seventy participants (33 males and 37 females) aged from 18 to 29 years (Median 22.00, IQR [21.00, 24.75] years) were enrolled in this RCT; 33 and 37 participants in the treatment and control groups respectively were evaluated and included in the analyses (Figure 4). Table 3 describes socio-demographic and clinical variables for the treatment and control groups. There was a balanced and homogenous distribution of these variables between the two groups. Figure 5 illustrates the mean and confidence intervals of 4 days postoperatively (3 measures per day) pain scores between study groups. Overall, pain scores of both groups were below average except for

the surgery day at 1pm. Also, pain scores in the control group exhibited a lower mean value throughout the study period, except for the morning of day 4 which the scores were higher. Interestingly, overall pain measures showed a circadian variation behaviour peaking at night. We examined the effect of NSAID (ibuprofen) chronotherapy treatment on postoperative pain scores in comparison to standard care regimen using LMMs (Table 4). We did not observe an effect of chronotherapy treatment on postoperative pain scores (estimate: 0.50, 95% CI = [-0.38, 1.39]). Slope differences between groups comparing pain scores change over day had an estimate of 0.0 (95% CI = [-0.23, 0.23]) and pain scores change over time had an estimate of 0.23 (95% CI = [-0.08, 0.56]).

Table 3: Socio-demographics and clinical parameters at baseline

Measurement	**Control (n=37)**	**Treatment (n=33)**
Age *(median [IQR])*	22.00 [21.00, 25.00]	22.00 [21.00, 24.00]
Gender *(%)*		
Female	19 (51.4)	18 (54.5)
Male	18 (48.6)	15 (45.5)
Oral Hygiene *(%)*		
Very good	9 (24.3)	3 (9.1)
Good	23 (62.2)	24 (72.7)
Poor	5 (13.5)	6 (18.2)
Smoking *(%)*		
Yes	6 (16.2)	3 (9.1)
No	31 (83.8)	30 (90.9)
Shisha *(%)*		
Yes	8 (21.6)	4 (12.1)
No	29 (78.4)	29 (87.9)
Education level *(%)*		
Did not complete high school	2 (5.4)	0 (0.0)
Graduated from high school	21 (56.8)	20 (60.6)
Graduated from college/university	14 (37.8)	13 (39.4)
Annual Income *(%)*		
Less than 12000 JD	6 (16.2)	4 (12.1)
12000 JD - 18000 JD	11 (29.7)	15 (45.5)
18000 JD or more	5 (13.5)	5 (15.2)
I don't know	14 (37.8)	9 (27.3)
Missing	1 (2.7)	0 (0.0)
Tooth Impacted *(%)*		
48	25 (67.6)	13 (39.4)
38	12 (32.4)	20 (60.6)
Impaction Type *(%)*		
Vertical	8 (21.6)	11 (33.3)
Mesio-angular	12 (32.4)	9 (27.3)

Disto-angular	2 (5.4)	1 (3.0)
Horizontal	13 (35.1)	11 (33.3)
Upside-down	0 (0.0)	1 (3.0)
Missing	2 (5.4)	0 (0.0)
Pre CRP *(median [IQR])*	0.95 [0.38, 2.40]	1.00 [0.48, 2.70]
Pre MID *(median [IQR])*	42.00 [40.00, 48.00]	42.00 [38.00, 48.00]
Pre DHC *(median [IQR])*	108.00 [102.00, 112.00]	105.00 [100.00, 113.00]
Pre DHS *(median [IQR])*	147.00 [138.00, 152.00]	142.00 [135.00, 155.00]
Pre DV *(median [IQR])*	106.00 [102.00, 111.00]	105.00 [98.00, 113.00]
Local Anesthesia Carpules- n *(median [IQR])*	2.00 [2.00, 3.00]	2.00 [2.00, 2.00]
Surgery Duration *(median [IQR])*	14.00 [9.00, 20.00]	16.00 [10.00, 20.00]
Lingual Retraction *(%)*		
Yes	18 (48.6)	18 (54.5)
No	18 (48.6)	15 (45.5)
Missing	1 (2.7)	0 (0.0)
Surgery Complication *(%)*		
Yes	2 (5.4)	2 (6.1)
No	35 (94.6)	31 (93.9)
Other Surgery Complications *(%)*		
1 mm of the root tip left in the ID canal	0 (0.0)	1 (3.0)
Facial palsy, disappeared later	1 (2.7)	0 (0.0)
Root tip	0 (0.0)	1 (2.8)
Tooth displacement (lingual pouch)	1 (2.7)	0 (0.0)
No complications	34 (91.9)	34 (94.4)
Pain Baseline *(median [IQR])*	0.00 [0.00, 1.00]	0.00 [0.00, 0.00]

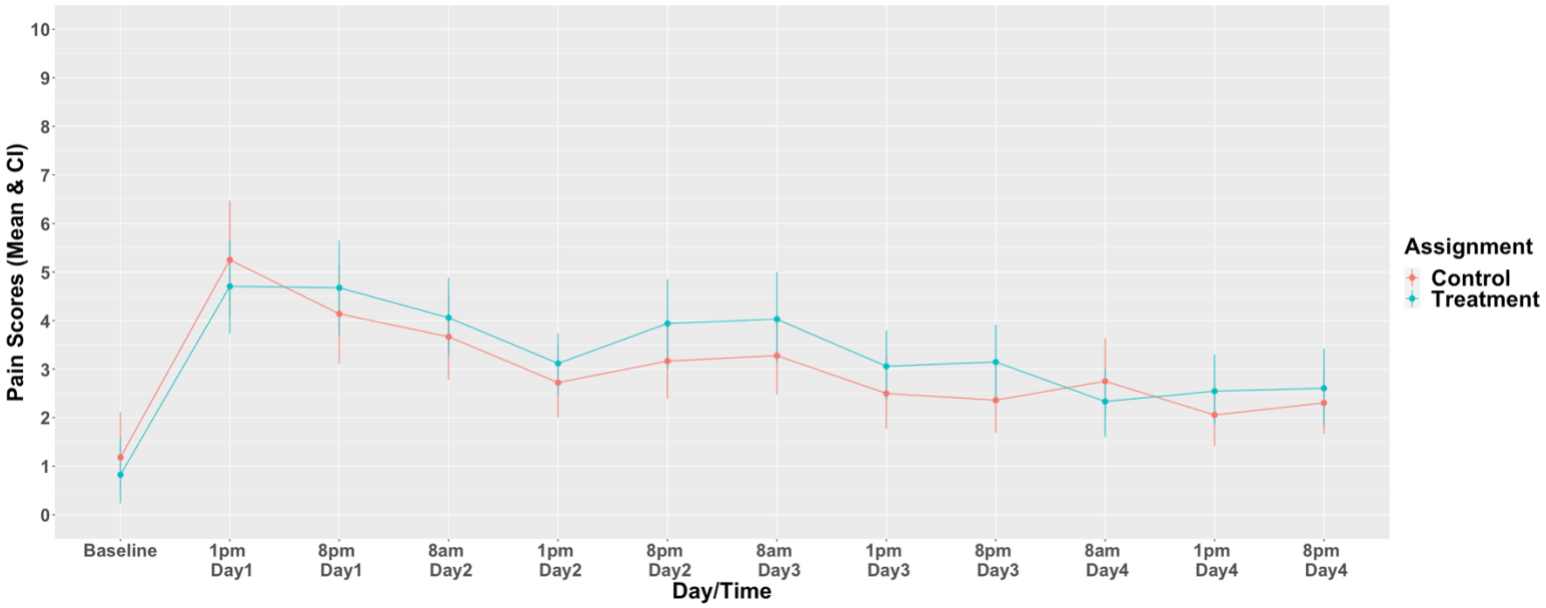


Figure 5: VAS pain scores between treatment and control groups

Table 4: Linear mixed effects model estimating NSAID chronotherapy treatment effect on postoperative VAS pain scores

	Estimate	**95% CI**	***p*-Value**
Intercept	1.46	(-1.17, 4.09)	0.277
Day	0.14	(-0.67, 0.97)	0.725
Time	0.95	(-0.04, 1.96)	0.062
Day:Time	-0.23	(-0.56, 0.08)	0.155
Treatment	0.50	(-0.38, 1.39)	0.266

CI = Confidence Intervals

Similarly, we did not observe any difference between pre- and post-operative CRP, facial swelling measurements for both groups (MID, DHS, DHC, DV) (Table 5). In addition, there was no difference between the groups regarding rescue medications (Supplemental Table 5).

Table 5: Pre- and Post-operative measurements

	Control (n=37)	Treatment (n=33)	p-Value [a]
Pre CRP *(median [IQR])*	0.95 [0.38, 2.40]	1.00 [0.48, 2.70]	0.605
Post CRP *(median [IQR])*	13.05 [4.58, 21.95]	12.18 [5.72, 24.26]	0.572
Pre MID *(median [IQR])* – ***mm***	42.00 [40.00, 48.00]	42.00 [38.00, 48.00]	0.571
Post MID *(median [IQR])* - ***mm***	25.00 [18.00, 31.00]	22.00 [18.00, 28.00]	0.544
Pre DHC *(median [IQR])* - ***mm***	108.00 [102.00, 112.00]	105.00 [100.00, 113.00]	0.571
Post DHC *(median [IQR])* - ***mm***	111.00 [106.00, 115.00]	110.00 [105.00, 116.00]	0.916
Pre DHS *(median [IQR])* - ***mm***	147.00 [138.00, 152.00]	142.00 [135.00, 155.00]	0.617
Post DHS *(median [IQR])* - ***mm***	151.00 [142.00, 156.00]	147.00 [144.00, 156.00]	0.663
Pre DV *(median [IQR])* - ***mm***	106.00 [102.00, 111.00]	105.00 [98.00, 113.00]	0.552
Post DV *(median [IQR])* – ***mm***	110.00 [106.00, 116.00]	108.00 [103.00, 115.00]	0.454

[a]Kruskal-Wallis rank-sum test; CRP, C-reactive protein; MID, maximum interincisal distance; DHC, horizontal distance to the corner; DHS, horizontal distance to the symphysis; DV, vertical distance

7.4 Discussion

To our knowledge, this is the first RCT to evaluate NSAIDs chronotherapy on post-operative pain and healing after a third molar surgical extraction. We did not observe any clinical and statistical difference in postoperative pain management and indicator of healing (e.g., edema and swelling) and inflammation (CRP serum levels) between the two groups. These findings could be explained by the fact that both inflammation and pain perception exhibit a circadian behavior; that is, when

the inflammatory pathway is triggered by a peripheral trauma or injury, proinflammatory mediators such as IL-1β, TNF-a and IL-6 are released with a peak during daytime [246, 247]. These mediators then stimulate CRP secretion and cause post-operative tissue edema [248]. Also, post-operative pain following surgical interventions is triggered by inflammatory mediators such as Bradykinin and PGE2 [249], and this inflammatory pain perception peaks during the active phase [92]. In other words, post-operative pain, swelling and CRP serum levels happen during the day, but not at night. This was clearly observed in our study as both treatment and control groups showed similar CRP serum levels, post-operative swelling measures and VAS pain scores, despite the fact that treatment group received a placebo pill during nighttime.

We have previously demonstrated pain and inflammation follow a circadian rhythm using a fracture model in mice. Our study concluded that NSAIDs administration during the active phase of the day results in improved post-operative recovery and pain management [239]. By contrast, NSAIDs taken during nighttime may impair bone healing [239].Interestingly, while we did not observe hindered healing in terms of post-operative swelling reduction, our RCT results showed that, despite the extra NSAID dose at nighttime among the control group, there was no difference in pain reduction between the two groups (Figure 5). This finding corroborates our hypothesis and implies that acceptable clinical results could be achieved with lower amounts of ibuprofen, which in turn could help minimize the complications associated with the use of ibuprofen such as heartburn and renal problems.

Another implication of our findings is that nocturnal pain might have a different origin than daytime pain. The pain scores in both groups seem to be peak at nighttime, but the fact that

nocturnal ibuprofen doses were ineffective indicate that that nocturnal pain might not involve inflammation. One explanation of such observation could be linked to the pain nature, which is of a neuropathic origin due to nerve inflammation following the surgery [250]. Similarly, to inflammatory pain perception that peaks during the day, neuropathic pain also exhibits a circadian rhythm often being worse at night [251]. Thus, in theory, we could replace the nighttime NSAIDs pill with Melatonin [252] and/or Gabapentin [253], which might be a better combined therapeutic approach for pain management.

There are several limitations to this RCT. First, the postoperative follow-up period was relatively short. With a follow-up period of 10 days or more participants would be fully recovered allowing a better post-operative assessment in their last recall visit. However, an extended follow up may increase drop out, compromising the study internal validity. While both post-operative CRP [142] and swelling [254] peak within 48-72h after the surgery, this study aimed to evaluate recovery duration rather than peak measurements of postoperative CRP or swelling. Second, two surgeons performed the surgical procedures. Although each one had different levels of surgical experience (one as a consultant OMF surgeon and the other as OMF fourth year resident), several quality control procedures were in place (e.g., detail protocol, training). Moreover, the duration of the surgeries was very similar among both professionals, and we did not observe difference the study outcomes between the surgeons (data not shown). Finally, there was an imbalance in tooth extraction location (Right and Left). While lower left impacted teeth are harder for right-handed surgeons to extract, Baqain et al. reported increased risk of post-operative infection (Alveolar Osteitis) in relation to surgeon handedness and tooth extraction side but they did not observe an increased risk of post-operative pain and trismus [255].

7.5 Conclusion

Post-operative pain exhibits a circadian rhythm peaking during the night and the chronotherapy of NSAIDs could be a potential therapeutic approach for pain management. Also, nighttime NSAIDs pill after surgical intervention had no effect on pain control. Finally, large multicentric RCTs should be carried to further confirm these findings, which could amend the current standard care regimen for NSAIDs.

Data Availability

Data and R Script are available upon request from the corresponding author.

Funding: This study was funded by Jordan University of Science and Technology in Irbid, Jordan (funding number: 20170307).

Ethics Declarations

Ethical approval:

The study was approved by the Research Ethics Committee on humans- Institutional Review Board (IRB) at Jordan University for Science and Technology and Jordanian Food and Drug Administration (JFDA). The trial was prospectively registered with the ClinicalTrials.gov, number NCT03789058.

Consent to participate:

Verbal and formal written consents were obtained from all participants. (copies of all the participants are available with the corresponding author).

Consent to publish:

Not applicable.

Conflict of interest:

The Authors declare no competing interests.

A preface to manuscript III

This manuscript investigates the effect of NSAIDs chronotherapy on pain management in impacted third molar extractions utilizing cross-over design.

8 Chapter 8: Manuscript III

Title: Effect of time-dependent ibuprofen administration on the post operatory after impacted third molar extraction: a cross-over randomized controlled trial

Fabián Pérez-González[1], Mohammad Abusamak[2], Luis Miguel Sáez-Alcaide[1], Jesus Torres García-Denche[1], and Faleh Ahmad Tamimi Marino[3]

1. Faculty of Dentistry, Department of Dental Clinical Specialties, University Complutense of Madrid, Plaza Ramón y Cajal S/N, 28040 Madrid, Spain
2. Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Canada
3. College of Dental Medicine, Qatar University, Doha, Qatar

Fabián Pérez-González and Mohammad Abusamak are the first co-authors.

Corresponding author:

Fabián Pérez-González, Faculty of Dentistry, Department of Dental Clinical Specialties, University Complutense of Madrid, Plaza Ramón y Cajal S/N, 28040 Madrid, Spain

Email: fabianpe@ucm.es

Published in the Oral and Maxillofacial Surgery Journal on August 3rd, 2022.

https://doi.org/10.1007/s10006-022-01104-8

Abstract

Purpose: To evaluate time-dependent administration of ibuprofen in a lower third molar extraction model.

Methods: Eleven patients requiring bilateral surgical removal of lower third molars were recruited and randomized into a blinded crossover randomized controlled trial. For 3 days after surgery, the control group was prescribed ibuprofen 400 mg every 8 h. On the other hand, the experimental group received also ibuprofen 400 mg at breakfast and lunch, replacing the dinner intake with a placebo. Pain measurements (Visual Analog Scale from 0 to 10) were recorded at baseline, 24, 48, and 72 h postoperatively. Facial swelling and trismus were also measured at baseline, 24, and 72 h postoperatively.

Results: Postoperative swelling and pain perception did not show significative difference between the control and experimental groups at 24, 48, and 72 h. Trismus was significantly lower in the control group than in the experimental group at 72 h postoperatively ($p = 0.008$). Rescue medication consumption seemed to be comparable between groups.

Conclusion: Eliminating nighttime ibuprofen might be insignificant for pain control after third molar extraction.

8.1 Introduction

Third molar (wisdom tooth) extraction, both prophylactically and due to odontogenic infection, is one of the most common surgical interventions in the world [256-259]. Postoperatively, such surgery is associated with pain, swelling, or/and trismus [256, 259-262]. Third molar extraction also causes high anxiety levels, increases morbidity, and impacts patients' quality of life [263].

In addition, wisdom teeth surgery is estimated to cost $3 billion per year in the USA alone, which places a burden on healthcare systems, and a proper pain management with a correct drug dosage could result in savings of $130 M per patient and reduce the back to work after health problems from 66 to 27% [263, 264].

Several medications are routinely prescribed for pain management after third molar extraction such as acetaminophen, opioids, and non-steroidal anti-inflammatory drugs (NSAID). Acetaminophen is insufficient for managing moderate to severe postoperative pain, and opioids can be addictive and cause constipation. NSAID, which are commonly prescribed, are indeed efficient in managing postoperative pain, but they have several adverse effects such as gastrointestinal complications and delayed bone healing [114, 208, 265]. Ibuprofen is the most used NSAID [266]. It is a 2-propionic acid derivate that was discovered in the 1960s by the British Boots Group, and it is a peripheral-acting analgesic with a high anti-inflammatory effect that reversibly inhibits COX-1 and COX-2 [210]. All organisms have a 24-h cycle pattern known as *circadian rhythm* that controls and modulates a wide range of their biological activities [225]. These rhythms have two main phases: an active or diurnal phase and a rest or nocturnal phase [267, 268]. The circadian rhythms also regulate the bone metabolism in 24-h oscillations affecting its formation, resorption [229, 230, 269], and even its healing after bone fracture [57, 269].

Moreover, inflammatory mediators have been described as day-time dependent [225, 228]. For example, pro-inflammatory cytokines such as IL-1 β and IL-6 are higher during the active phase, while the anti-inflammatory cytokines reach their peak during the resting phase [237, 270]. Further studies suggested that cytokines play a major role in pain perception, and thereafter, pro-inflammatory cytokine modulation is vital for pain management and tissue recovery [271].

Different clinical studies suggested that timing of drug administration (i.e., *chronotherapy*) with the circadian rhythm of the body could lead to superior pain management and fewer side effects [272]. For example, acetaminophen action is better during the night, and NSAIDs absorption and its anti-inflammatory effect is higher when it is administered during the active phase of the circadian rhythms [273]. In fact, it has been shown that NSAID intake during the active phase (diurnal) could modulate the synthesis and release of cytokines by the cyclooxygenase inhibition, causing a decrease in the pro-inflammatory cytokines (like IL-1 β) and promoting the anti-inflammatory cytokines (such as IL-13 and IL-14) [93]. Furthermore, COX-1 and COX-2 activity follows a circadian pattern that regulates bone metabolism after bone tissue damage. Therefore, understanding these circadian variations in NSAID pharmacokinetics (absorption and maximal effect), cytokine release and COX activity would imply the possibility of establishing a chronotherapeutic treatment that would maximize the effect of NSAIDs while reducing their postoperative side effect [91, 237, 274]. This was clearly demonstrated in an animal study evaluating NSAID chronotherapy after bone fracture in mice. They concluded that NSAID administration during the active phase revealed a superior bone healing outcome, while the administration during the rest phase showed a prolonged inflammatory phase, subsequently decreasing postoperative recovery [93].

In the recent years, it has been proposed that the pharmacokinetic of these drugs could be influenced by the administration time. This implies that coordinating the drug intake time with the circadian clock, a.k.a. *chronotherapy*, could help improve treatment effectiveness.

This pilot study aims to evaluate the effect of NSAID *chronotherapy* in postoperative recovery after third molar extraction related to the swelling, trismus, and pain scores, compared to a conventional NSAID administration regimen. The main hypothesis is that NSAID dosage according to the circadian rhythms results in a similar or better postoperative recovery.

8.2 Material and methods

8.2.1 Study design

This randomized, double-blinded, placebo-controlled, crossover design pilot study was conducted following the CONSORT [275] guidelines and was carried out at the Oral Surgery Department of the Faculty of Dentistry at the Complutense University of Madrid (UCM). The study was evaluated and approved by the Research Ethics Committee at the San Carlos Clinical Hospital of Madrid, Spain (Trial registration code CEIC 19/216-R_M_BNI.) and the *Agencia Española del Medicamento y Productos Sanitarios* (AEMPS: *Spanish Agency of Drugs and Sanitary Products*, EUDRACT number 2019–000,736). Informed consent was obtained from all participants in writing prior to conducting the research, and the principles of the Declaration of Helsinki for research involving human subjects were followed. The trial was registered with the ClinicalTrials.gov number NCT05126264 in October 2021.

8.2.2 Participants and inclusion criteria

Patients were accepted in the clinical trial from those who attended the Oral Surgery Department at the Dentistry Faculty of the Complutense University of Madrid between September 2019 and December 2020 with needs of lower third molar extraction. The screening examination was performed by a 2nd-year resident program in Oral Surgery and Implant Dentistry (FPG) and included a medical and dental questionnaire and a standardized panoramic radiograph made at the Dental Radiology Service, Faculty of Dentistry, Complutense University of Madrid (CS 9300®, Carestream Dental, Atlanta, GA, USA). Healthy males and females aged between 18 and 35 years old who presented with impacted bilateral lower third molar with similar surgical difficulty [276] were included in our study. Patients who refused to give consent, to undergo surgery, or who were unable to return for evaluation were excluded. In addition, patients with a history of gastrointestinal disease, pregnant or lactating, and with active periodontal disease were excluded.

8.2.3 Blinding and randomization

To reduce bias for this pilot study, both patients and surgeons were blinded to treatment group allocation. Second-year surgical resident performed all the surgical procedure (FPG). Both third molar side and medication (3 times ibuprofen or 2 times ibuprofen + placebo) randomization were performed by the main investigator (JTGD) using a coin flip. Other than providing medications, the investigator did not have any contact with the study's participants or involvement in data collection. Data analysis was performed by an independent investigator (MA).

8.2.4 Surgical procedure

All surgical procedures were conducted by a single surgeon (FPG) at 9:00 am in all the cases. A minimum of 1 month for washing up was left between one surgery and the intervention on the contralateral side. Local anesthesia consisting of 4% articaine with adrenaline 1:100,000 was

administered for the inferior alveolar, lingual, and buccal nerves (Ultracaine®, Normon SL, Madrid, Spain). An intrasulcular incision from the lower first molar with a vertical releasing incision in the ramus was made, and then a mucoperiosteal flap was elevated. A tungsten carbide bur with a surgical handpiece was used to perform bone removal, and when necessary, also a Lindemann bur was used to section the third molar. After the tooth extraction, bony edges were smoothened, and socket was washed with copious use of saline solution. Then, the flap was sutured with simple interrupted sutures using 4.0 Supramid (Proclinic®, Zaragoza, Spain). Surgery time, surgery difficulty according to Parant scale [276], and surgical complications were also recorded. Patients were prescribed amoxicillin 750 mg to be taken every 8 h for 7 days postoperatively. The control group received one 400 mg ibuprofen capsule every 8 h for 5 days, while the experimental group received one 400 mg ibuprofen capsule in the morning and evening and one placebo capsule in the night for 5 days.

8.2.5 Study outcome measures

According to our primary outcome, pain measurements (Visual Analog Scale from 0 to 10) were recorded at baseline, 24, 48, and 72 h postoperatively. As a secondary outcome, facial swelling parameters such as distance from Tragus to Pogonin (Tg-Pg) [277] and trismus (i.e., interincisal distance) were evaluated at baseline, 24, and 72 h postoperatively. The rescue medication (RM) (acetaminophen 650 mg) consumed by patients was also recorded (day/time/number of pills).

8.2.6 Statistical analysis

Data were entered on a spreadsheet (MS Excel 2007, Microsoft Inc., Redmond, WA, USA) until the end of the trial and analyzed with R statistical program (4.0.2) by an independent investigator (MA). The significance level chosen for all statistical tests was $p < 0.05$. Descriptive statistics were

calculated for all variables (frequency, median, and IRQ range). After testing for normality, non-parametric tests were used. For the quantitative variables, Wilcoxon signed rank test was conducted, and as to qualitative variables, McNemar's chi-squared test was performed.

8.3 Results

In this RCT, 11 patients, median age of 21 years (*IQR* = 20.00, 21.50) were recruited, evaluated, and included in our analysis. Baseline parameters are presented in Table 6. With the exception of extracted tooth, impaction type, and surgery duration, all demographic variables seem to be equally distributed among experimental groups, as this study followed a split-mouth model. From the sample, 10 patients were Caucasic, and only one patient was Asiatic. Tooth number 48 was predominantly extracted in the control group (10/11 cases). On the other hand, surgeries lasted longer in the treatment group. Almost all surgeries were performed on partially impacted teeth (10/11 cases). Medical history and medication were not determined for the study.

Table 6: Demographics and baseline parameters

Variables	**Treatment**	**Control**
Position Winter (%)		
Distal	2 (18.2)	0 (0.0)
Horizontal	3 (27.3)	2 (18.2)
Mesial	5 (45.5)	4 (36.4)
Vertical	1 (9.1)	5 (45.5)
Surgery Description (%)		
I – Flap only	0 (0.0)	1 (9.1)
II – Flap and osteotomy	7 (63.6)	7 (63.6)
III – Flap, osteotomy, and coronal sectioning	3 (27.3)	1 (9.1)
IV – Flap, osteotomy, and coronal and radicular sectioning	1 (9.1)	2 (18.2)
Swelling Baseline (median [IQR]) **-mm**	151.00 [146.50, 160.00]	152.00 [150.50, 160.00]
Trismus Baseline (median [IQR]) **-mm**	45.00 [40.00, 55.50]	45.00 [41.00, 53.50]
VAS Pain Baseline (median [IQR])	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]
Surgery Duration (median [IQR]) **-mins**	13.45 [11.57, 15.00]	9.06 [6.36, 11.35]
Quirurgical Complications (%)		
Flap tear	0 (0.0)	1 (9.1)
No	11 (100.0)	10 (90.9)
Postoperative Complications (%)		
No	10 (90.9)	11 (100.0)
Temporary paresthesia buccal nerve	1 (9.1)	0 (0.0)

Table 6 presents postoperative clinical variables. Regarding our primary outcome, there was no significant difference of VAS pain scores between groups at 24 h, 48 h, and 72 h postoperatively ($p > 0.05$) (Figure 6). All patients reported that they stopped feeling pain between day 3 and day 5 after surgery with no significant difference between groups ($p > 0.05$). Similarly, we also found no significant difference in total RM consumption ($p > 0.05$). In accordance with our secondary outcome, both facial swelling and mouth opening measures (24 h and 72 h postoperatively) were similar in experimental groups (Figs. 7 and 8, respectively). However, patients in the control group had less restricted mouth opening 72 h postoperatively in comparison to the treatment group ($p = 0.008$). No pharmacological side effects were reported in the study.

Table 7: Post-Operative Clinical Parameters

Variables	**Treatment**	**Control**	**P***
n	11	11	
Primary Outcome			
VAS Pain 24h (median [IQR])	5.00 [4.00, 6.50]	4.00 [3.50, 6.00]	0.669
VAS Pain 48h (median [IQR])	3.00 [2.00, 5.00]	3.00 [2.00, 5.00]	1
VAS Pain 72h (median [IQR])	3.00 [0.50, 3.00]	1.00 [1.00, 3.00]	0.510
Secondary Outcome			
Swelling 24h (median [IQR]) **-mm**	156.00 [154.50, 167.50]	163.00 [155.00, 167.50	0.929
Swelling 72h (median [IQR]) **-mm**	160.00 [152.00, 167.00]	160.00 [154.50, 166.00]	0.624
Trismus 24h (median [IQR]) **-mm**	30.00 [22.50, 31.50]	31.00 [22.50, 34.00]	0.823
Trismus 72h (median [IQR]) **-mm**	25.00 [22.50, 33.00]	36.00 [27.50, 43.00]	**0.008**
RM Consumption			
Total RM pills (median [IQR])	1.00 [0.00, 2.50]	0.00 [0.00, 1.00]	0.396
Other Pain Measures			
No Pain – Day (median [IQR])	4.00 [3.50, 5.00]	5.00 [3.50, 5.00]	0.529

* Wilcoxon signed rank test RM: Rescue Medication

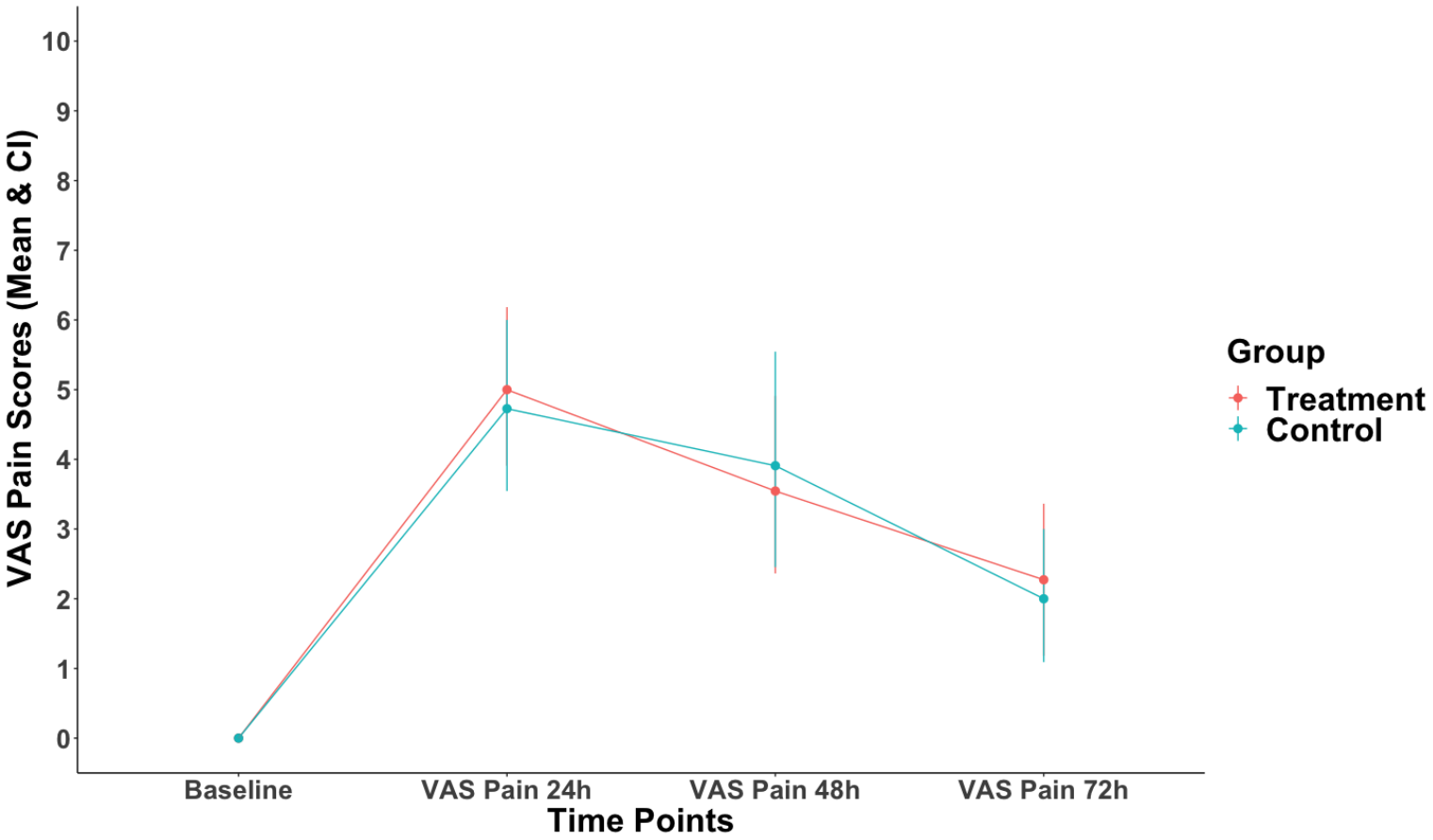


Figure 6: Postoperative VAS pain measures between treatment and control groups

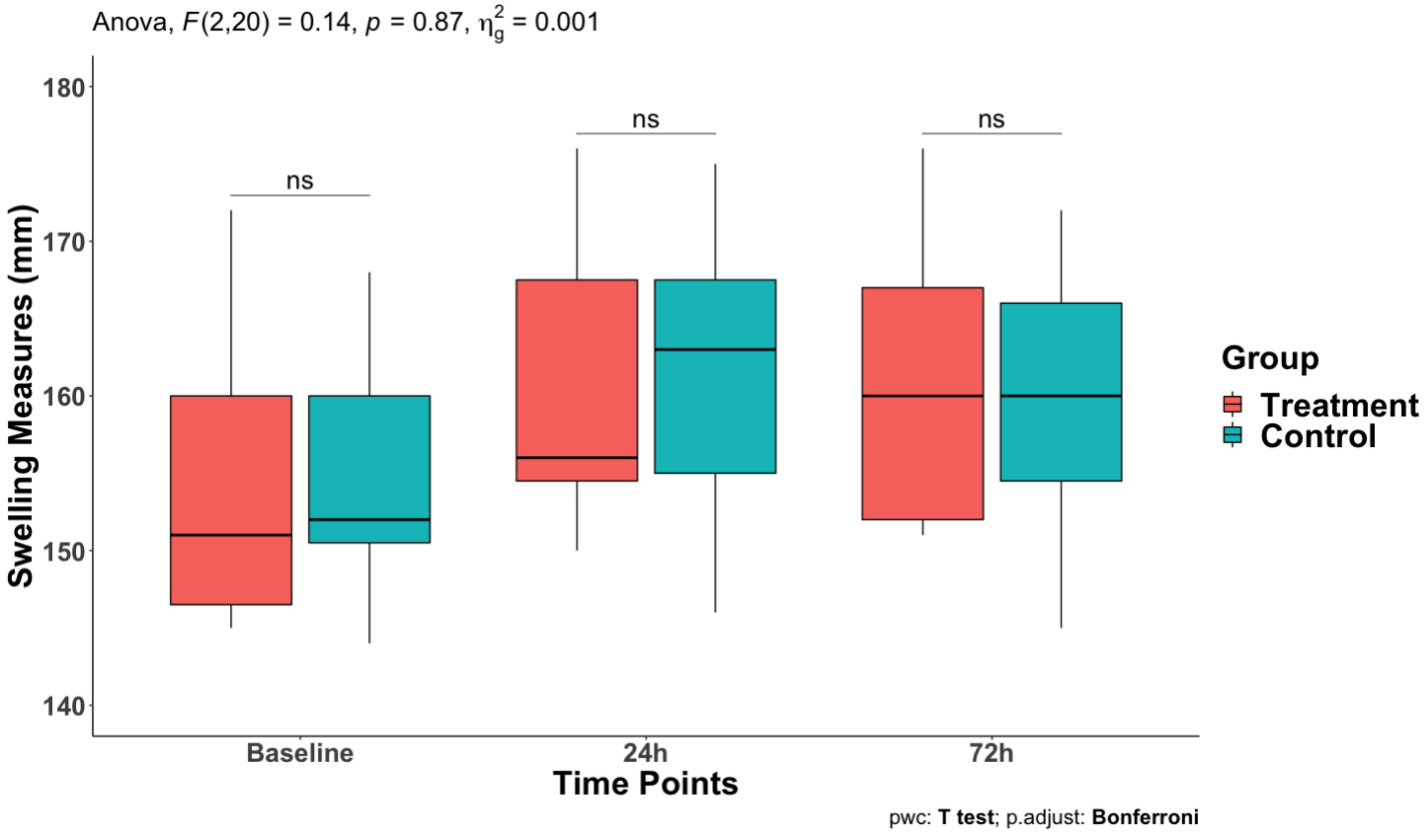


Figure 7: Postoperative swelling repeated measures boxplot between treatment and control groups

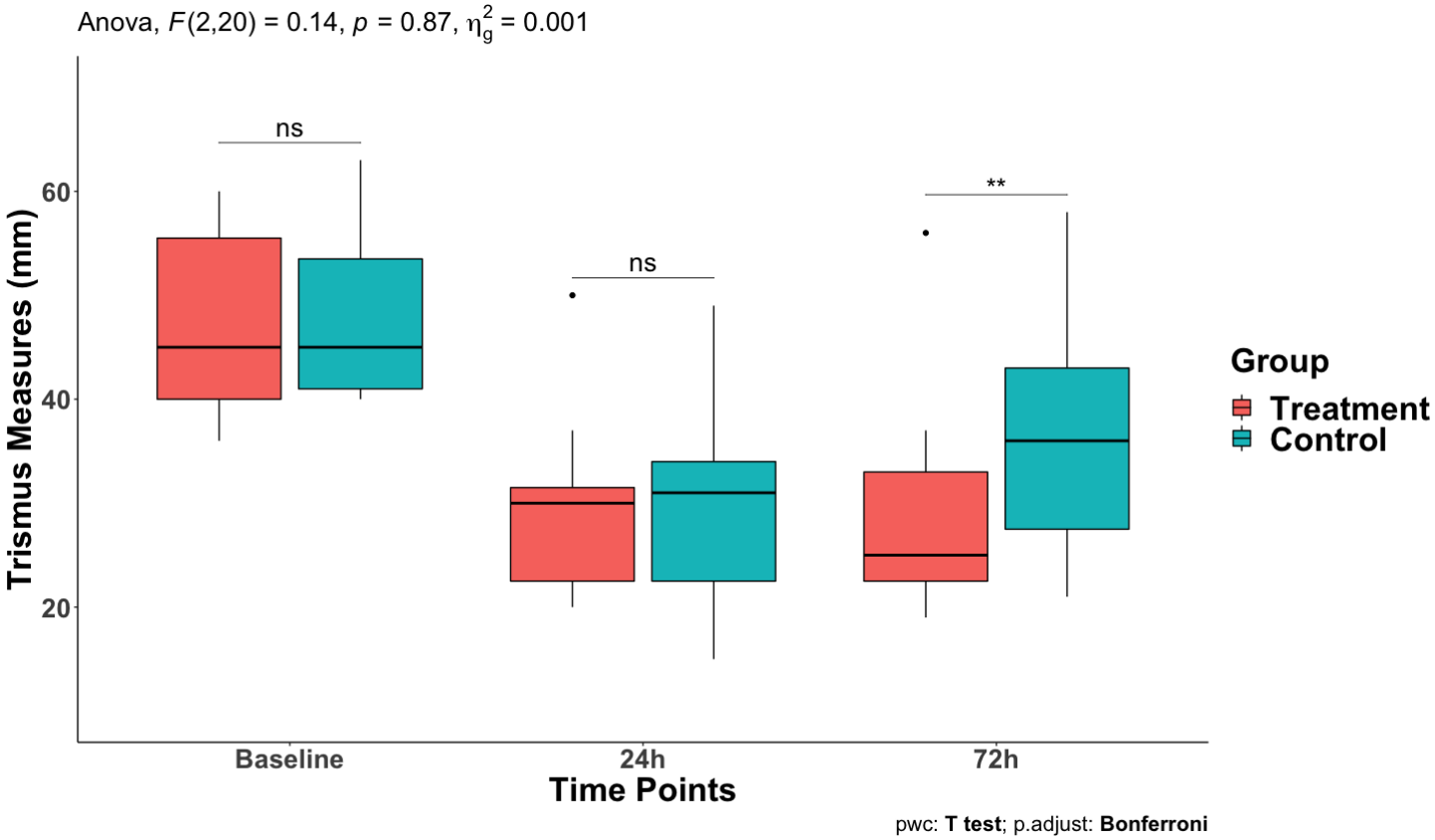


Figure 8: Postoperative swelling repeated measures boxplot between treatment and control groups

8.4 Discussion

The aim of this pilot study was to evaluate the effect of the NSAID chronotherapy in the postoperative period after surgical extraction of third molars, comparing swelling, trismus, and pain.

As it is known, chronobiology is a medical discipline that was first described in the 1960s decade. Its study involves the body rhythms that regulate different functions and are known as circadian rhythms [278, 279]. These circadian rhythms are 24-h cycles that modified the human body

condition according to the function and daytime, in two different phases: an active or diurnal and a breaking or nocturnal phase [225].

The influence of circadian rhythms on metabolic processes and their ability to modify the pharmacokinetics of drugs has been described. They can modify the efficacy or side effects of drugs depending on the time of day they are taken; this phenomenon is known as chronotherapy [272]. Chronotherapy has been shown to be efficient in different medical conditions by matching drug administration with circadian rhythms. For example, in the hypertension treatment, the nocturnal intake of the drug has demonstrated to reduce cardiovascular risk [280, 281]; or in oncologic patients, the radiotherapy effect is more efficient, and the side effects are fewer when it is administered during the morning [282]. More recently, it has been hypothesized that the regulation of anti-inflammatory drugs in SARS-CoV-2 patients according to circadian rhythms could result in beneficial management of these patients [156]. Also, one study on asthma patients treated with corticosteroids showed that its administration in the evening provide benefits compared to the morning administration [283].

Lower third molar surgery (LTMS) is, irremediably, associated with pain, swelling, or/and trismus [256]. These postoperative complications can be treated with a wide range of drugs including analgesic and antipyretic (acetaminophen), NSAID (ibuprofen, dexketoprofen), corticosteroids, and opioids [284]. Therefore, many studies have focused on evaluating what would be the best therapeutic option in these cases.

Regarding the trismus associated with postoperative LTMS, Saez et al. compared a chitosan gel against a placebo, observing a lower limitation in the experimental group[285], unlike other

authors who used cryotherapy [286], or herbal extracts composed of drugs [287], who did not observe differences between the groups. This clinical trial reported differences significantly lower in the control group than in the experimental group at 72 h postoperatively ($p = 0.008$).

Swelling appears in the soft tissues after an injury or an intentional aggression as the LTMS, according to the immunity response. The tissular damage promotes the prostaglandins releasing and facilitating macrophages or interleukin appearance which works on tissular damage reparation and pain management [288]. There is no evidence about the role of chronotherapy on the swelling or pain after LTM extraction, but the literature is extensive comparing different drugs, dosages, or even combination of different drugs: for example, it is seen that the combination of ibuprofen with acetaminophen report a better post operatory in terms of swelling or pain sensation comparing to a placebo or drugs prescribed isolated [114, 289, 290]. This pilot study, with an alternative ibuprofen administration considering the circadian rhythms, had a positive effect in swelling and pain management, finding no statistical differences about the experimental and the control group in the immediate post operatory and 72 h. Under experimental conditions, the pain sensitivity is higher during the afternoon hours, so that the ibuprofen administration according to the chronotherapy with the morning and afternoon dosage should be enough to maintain a prolonged analgesia, reaching it maximum concentration in plasma when inflammatory mediators reach its peak [156].

Recently a similar study was performed comparing the effect of chronotherapy of the NSAIDs, finding no differences in terms of trismus or swelling indicator and pain scores between the experimental and the control group. They also considered that the night intake of NSAID do not provide any potential benefit in the post-surgical lower third molar treatment [180].

Nevertheless, in the present pilot study, the patients referred a higher pain intensity during the morning or the first hours in the afternoon. This difference was statistically significative ($p < 0.05$) in the experimental group, and it could hypothesize that the patients who did not received the NSAID in the nighttime suffered a higher accumulation of inflammatory mediators that could be traduced in a higher morning pain intensity. Also, though the time between both extraction is 1 month, according to the US Food and Drug Administration, 1 week is time enough for body washing up [291].

One of the strengths of this study is the split-mouth design, thus avoiding interindividual bias in the assessment of the different parameters studied; however, although the results observed in this study are positive, there are some limitations such as the randomization process and the necessity of implement a higher sample size to guarantee the results. Moreover, it should be interesting to compare the chronotherapy by investigating the inflammatory markers levels in blood samples and different types of NSAID with other dosages.

8.5 Conclusion

The use of ibuprofen mediated by chronotherapy has shown similar results to the classical dosage in terms of pain, swelling, or trismus after surgical third molar extraction.

Data availability

Data and R Script are available upon request from the corresponding author.

Funding

Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Contributions

F.P.G: design of the work, drafting of the work, conception, acquisition of data, and final approval of the version.

M.A: design and drafting of the work, interpretation of data, and final approval of the version.

L.M.S.A: final approval of the version.

J.T.G.D: design of the work and final approval of the version.

F.A.M.T: design of the work and final approval of the version.

Corresponding author

Correspondence to Fabián Pérez-González.

Ethics declarations

Ethical approval

The study was evaluated and approved by the Research Ethics Committee at the San Carlos Clinical Hospital of Madrid, Spain (Trial registration code CEIC 19/216-R_M_BNI.) and the Agencia Española del Medicamento y Productos Sanitarios (AEMPS: Spanish Agency of Drugs and Sanitary Products, EUDRACT number 2019–000736).

Consent to participate and publish

Informed consent was obtained from all participants in writing form.

Conflict of interest

The authors declare no competing interests.

Additional information

Publisher's Note

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

Fabián Pérez-González and Mohammad Abusamak are the first authors.

9 Chapter 9: Discussion and Conclusions

9.1 General discussion

My thesis had two aims: (1) to investigate the applications of chronotherapy in dentistry, and (2) to evaluate NSAIDs chronotherapy in the postoperative management of third molar surgical extraction. To address the first aim of my thesis, I conducted a scoping review to systematically map the applications of chronotherapy in dentistry. Overall, the scoping review showed promising clinical benefits when utilizing chromotherapy in various fields of dentistry. For example, treatment response and adverse events from chemotherapy and/or radiotherapy in head and neck cancer treatments was found to be time-dependent, and chronotherapy in head and neck cancer treatment could achieve superior clinical outcomes. However, these results should be interpreted with caution as current studies showed methodological limitations that could be optimized in future clinical trials. These findings were in accordance with two recently published systematic reviews evaluating chronotherapy in cancer treatments. Shuboni-Mulligan et al. 2019 in their review evaluating the clinical impact of radiotherapy treatment time-of-day in various cancers [282] concluded that radiation chronotherapy could reduce adverse events in comparison to non-time-stipulated radiotherapy [282]. The other review, by Printezi et al. 2022, evaluated the toxicity and efficacy of chronotherapy in chemotherapies for different tumors [292]. They reported that while the efficacy of chrono-chemotherapy was maintained, there was evidence of reduced toxicities in comparison to non-time-stipulated chemotherapy treatments [292]. However, both of these systematic reviews agreed that optimized study designs with larger sample size are needed to amend clinical practice guidelines.

Furthermore, the benefits from understanding the influence of the circadian clock has on bone physiology in the field orthodontics have demonstrated. Studies included in the scoping review, which investigated time-dependent force application aligned with the circadian rhythm, demonstrated potential to achieve superior tooth movement and bone remodeling. However, these studies were only experimental and conducted in animal models more than 20 years ago. To this day, no clinical trials have investigated similar outcomes despite their promising results. Interestingly, there is an increasing body of literature linking different circadian clocks to osteointegration of dental implants [293-295]. It has been reported that the titanium surface of dental implants modulates specific clock genes. NPAS2 and PER2 seem to facilitate osteointegration and Vitamin D and Melatonin supplements can directly improve osteointegration as well [293-295]. To summarize, expanding our understanding of the chronobiology of bone could result in better treatment quality in orthodontics and dental implants.

The second aim of my thesis was to evaluate NSAIDs chronotherapy on post-operative recovery after third molar extraction. To address this aim, we conducted two pilot RCTs with two different designs (Parallel vs Cross-over). These two studies were built on our preclinical study evaluating NSAIDs chronotherapy on post-operative recovery in animal fracture model, showing that restricting the intake of NSAIDs to the active phase in mice achieves faster recovery and superior pain control[15]. Accordingly, we evaluated the effect of daytime NSAIDs consumption as opposed to traditional NSAIDs administration regimen (three times a day) for pain management following surgical extraction of third molars. Both RCTs showed evidence of similar postoperative recovery (pain perception and facial swelling and edema) despite the fact that the chronotherapy group had reduced NSAIDs intake overall each day postoperatively. Our findings are in fact

aligned with the literature. First, perceived pain seemed to increase during the night in both therapeutic regimens, which could be explained by the fact that post-operative pain peaks during the day and troughs during the night suggesting that a nighttime dose of NSAIDs might be ineffective [78]. One explanation of this observation could be linked to the nature of pain, which is of a neuropathic origin due to nerve inflammation following the surgery [250]. Similarly, to inflammatory pain perception that peaks during the day, neuropathic pain also exhibits a circadian rhythm often being worse at night [251]. Second, inflammation also exhibit a circadian behavior, that is, when the inflammatory pathway is triggered by a peripheral trauma or injury, proinflammatory mediators such as IL-1β, TNF-a and IL-6 are released with a peak during daytime [246, 247]. These mediators then stimulate CRP secretion and cause post-operative tissue edema [248]. These observations could explain the similarity of post-operative facial swelling measurements between experimental groups.

9.2 Strengths and limitations

There are several limitations of my thesis that should be considered. For manuscript I, while scoping reviews are meant to systematically map the available literature and identify gaps in the knowledge underpinning chronotherapy in dentistry, the results should be interpreted with caution. Because scoping reviews are not designed to answer a narrow question about efficacy or reduced side effects of certain treatments or conditions that might amend current clinical practice guidelines. However, I conducted a blind risk of bias assessment which allowed me to better understand the quality of the included studies that was reflected in the conclusions. Further, the scoping review included animal studies whose findings can be hard to directly translate to patients. But when I consider the scarcity of literature on the subject, it was useful to see what has been

reported in both animals and humans studies which would help other researchers produce systematic reviews for them in the future. Further, there are topics that were studied in animals but not in humans; thus, by adding the animal component, I was able to indicate the areas that would need further clinical studies.

To fulfill objectives 2 and 3 we used two different study RCT designs, which have also several limitations. First, both studies followed up patients for no more than 3 days post-operatively. Consequently, post-operative VAS pain scores and facial swelling did not return to baseline measures. This is important to evaluate chronotherapy regimen in the context of post-operative recovery, whether it will be prolonged or shortened. Second, while the control groups had a placebo pill replacing the nighttime NSAIDs (ibuprofen), the total dosage was different between groups. That is, the chronotherapy groups received 800 mg (at 8 AM and 1 PM) and the control groups 1200 mg (at 8 PM, 1 PM and 8 PM). Therefore, it may be argued that we cannot test the effect of the time on the outcomes of interest. However, after oral administration of Ibuprofen, peak serum concentration is achieved in 1 to 2h, and it has a half-life of approximately 2h [240]. After 4-5 half-lives (8-10h), ibuprofen is eliminated from our system, as its plasma concentration is not considered to be clinically effective [296]. Thereafter, two assumptions could be attained; First, by the time that both groups take their morning dose (8 AM), there should be no residual ibuprofen in their system. Second, during nighttime (8 PM to 8 AM), only the control group has effective plasma concentration of ibuprofen. Therefore, both chronotherapy and control groups have similar ibuprofen serum concentration during the day but not during the night. In turn, this corroborates the idea that pain during the night could be of a different origin that renders ibuprofen ineffective.

Another limitation is the sample size calculation and the power of both clinical studies. When considering these surgical models to evaluate post-operative healing outcomes implementing chronotherapy, there are no studies available investigating repeated measures of pain scores and NSAIDs chronotherapy. Consequently, it was a challenge to obtain accurate estimates needed for the sample size calculation. However, the purpose of these pilot studies not only serves as a proof of concept, but also provides us with more accurate data for robust sample size calculations for future studies. Indeed, the next step would be an optimized non-inferiority randomized clinical trial that could result in amending existing clinical guidelines for pain management after surgeries. Further, since VAS pain scores are patient self-reported outcome measures, in the cross-over design, patients who experienced much difficult extraction or a simpler one in the first period, their pain scores will be greatly influenced in the second period (i.e., response shift). Since patient-reported pain might be incorrectly interpreted if the response shift is not taken into consideration, the then-test, a retrospective test, could be used to recalibrate patients' pain scores during their follow up visits [297]. These recalibrated pain scores could be used to evaluate NSAIDs chronotherapy in pain management post-operatively in future studies.

Moreover, although there were multiple surgeons with different levels of experience performing the surgical extraction of third molars, this was mitigated by several quality control procedures such as detailed protocols and training. In addition, there was a discrepancy in tooth extraction location (right vs left) between participants in both studies. However, evidence suggest that the tooth extraction location might be associated with increased risk of post-operative infection (Alveolar Osteitis), but it is not associated with increased risk of post-operative pain and trismus

[255]. Despite the abovementioned limitations, VAS pain scores and facial swelling did not clinically or statistically differ across groups post-operatively, and these findings were consistent in both study designs.

9.3 Significance and knowledge translation

My PhD work contributes to the body of literature in two main areas. Firstly, by conducting the scoping review, I summarized the available literature in dentistry supporting chronotherapy applications in dental practice. Also, I evaluated the evidence thus far underlining areas where further research is needed and demonstrated their potential benefits in improving patients' quality of life. Secondly, my research might contribute to developing a safe non-opioid approach to enhance postoperative recovery and bone healing in craniofacial and musculoskeletal injuries. Through a holistic understanding of the chronobiology of inflammation and pain, I could provide an accessible, simple, and effective pain control regimen as well as actively contributing to the effort to resolve the worldwide opioid crisis and addiction.

9.4 Future research directions

The scoping review revealed that it is feasible to further investigate chronotherapy in head and neck cancer. This could be achieved by conducting a systematic review and meta-analysis testing chrono-chemotherapy and chrono-radiotherapy on head and neck carcinomas in terms of treatment response and/or reduced toxicities.

Our findings from the RCTs showed that the nighttime administration of NSAIDs might not be effective in pain management after surgery. Therefore, the next step would be to conduct a non-

inferiority RCT, which eventually could change the current standard of care. Further, a replacement pill for nighttime NSAIDs could be proposed and possibly patent for superior pain management regimen.

10 Chapter 10: Conclusion

- The scoping review demonstrated that chronotherapy has promising therapeutic benefits in various fields of dentistry, especially head and neck cancer treatments.
- Chronotherapy in dentistry, thus far, has been investigated in six areas namely, chemotherapy and radiotherapy, oral medicine, removable prosthodontics, post-operative pain management, local anesthesia, and orthodontics.
- Postoperative pain exhibits a circadian rhythm peaking during the night, and the chronotherapy of NSAIDs could be a potential therapeutic approach for pain management.
- The nighttime NSAIDs pill after the surgical intervention had no effect on pain control.
- Large multicentric RCTs should be carried to further confirm these findings, which could amend the current standard care regimen for NSAIDs.

11 Chapter 11: References

1. Dallmann R, Brown SA, Gachon F. Chronopharmacology: new insights and therapeutic implications. Annual review of pharmacology and toxicology. 2014;54:339-61.

2. Chen S, Fuller KK, Dunlap JC, Loros JJ. A pro-and anti-inflammatory axis modulates the macrophage circadian clock. Frontiers in immunology. 2020;11:867.

3. Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proceedings of the National Academy of Sciences. 2009;106(11):4453-8.

4. Parsons MJ, Moffitt TE, Gregory AM, Goldman-Mellor S, Nolan PM, Poulton R, Caspi A. Social jetlag, obesity and metabolic disorder: investigation in a cohort study. International Journal of Obesity. 2015;39(5):842-8.

5. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. Nature Reviews Cancer. 2003;3(5):350-61.

6. Bass J, Lazar MA. Circadian time signatures of fitness and disease. Science. 2016;354(6315):994-9.

7. Hastings MH, Goedert M. Circadian clocks and neurodegenerative diseases: time to aggregate? Current opinion in neurobiology. 2013;23(5):880-7.

8. Shilts J, Chen G, Hughey JJ. Evidence for widespread dysregulation of circadian clock progression in human cancer. PeerJ. 2018;6:e4327.

9. Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature. 2010;466(7306):627-31.

10. Panda S. Circadian physiology of metabolism. Science. 2016;354(6315):1008-15.

11. Baiju R, Peter E, Varghese N, Sivaram R. Oral health and quality of life: current concepts. Journal of clinical and diagnostic research: JCDR. 2017;11(6):ZE21.

12. Wirz-Justice A, Benedetti F. Perspectives in affective disorders: Clocks and sleep. European Journal of Neuroscience. 2020;51(1):346-65.

13. Bowles N, Thosar S, Herzig M, Shea S. Chronotherapy for hypertension. Current hypertension reports. 2018;20(11):1-24.

14. Thoonkuzhy C, Rahman M. New Insights on Chronotherapy in Hypertension: Is Timing Everything? Current hypertension reports. 2020;22(4):1-6.

15. Al-Waeli H, Nicolau B, Stone L, Abu Nada L, Gao Q, Abdallah M, et al. Chronotherapy of non-steroidal anti-inflammatory drugs may enhance postoperative recovery. Scientific reports. 2020;10(1):1-14.

16. Battaglin F, Chan P, Pan Y, Soni S, Qu M, Spiller ER, et al. Clocking cancer: The circadian clock as a target in cancer therapy. Oncogene. 2021;40(18):3187-200.

17. Rahman S, Wittine K, Sedić M, Markova-Car EP. Small molecules targeting biological clock; A novel prospective for anti-cancer drugs. Molecules. 2020;25(21):4937.

18. Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. Annual review of neuroscience. 2012;35:445.

19. Cao X, Yang Y, Selby CP, Liu Z, Sancar A. Molecular mechanism of the repressive phase of the mammalian circadian clock. Proceedings of the National Academy of Sciences. 2021;118(2):e2021174118.

20. Solt LA, Kojetin DJ, Burris TP. The REV-ERBs and RORs: molecular links between circadian rhythms and lipid homeostasis. Future medicinal chemistry. 2011;3(5):623-38.

21. Kojima S, Shingle DL, Green CB. Post-transcriptional control of circadian rhythms. Journal of cell science. 2011;124(3):311-20.

22. Reischl S, Kramer A. Kinases and phosphatases in the mammalian circadian clock. FEBS letters. 2011;585(10):1393-9.

23. Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science. 2009;326(5951):437-40.

24. Gallego M, Virshup DM. Post-translational modifications regulate the ticking of the circadian clock. Nature reviews Molecular cell biology. 2007;8(2):139-48.

25. Tamaru T, Hirayama J, Isojima Y, Nagai K, Norioka S, Takamatsu K, Sassone-Corsi P. CK2α phosphorylates BMAL1 to regulate the mammalian clock. Nature structural & molecular biology. 2009;16(4):446-8.

26. Yoshitane H, Takao T, Satomi Y, Du N-H, Okano T, Fukada Y. Roles of CLOCK phosphorylation in suppression of E-box-dependent transcription. Molecular and cellular biology. 2009;29(13):3675-86.

27. Luciano AK, Zhou W, Santana JM, Kyriakides C, Velazquez H, Sessa WC. CLOCK phosphorylation by AKT regulates its nuclear accumulation and circadian gene expression in peripheral tissues. Journal of Biological Chemistry. 2018;293(23):9126-36.

28. Ribeiro RF, Cavadas C, Silva MMC. Small-molecule modulators of the circadian clock: Pharmacological potentials in circadian-related diseases. Drug Discovery Today. 2021;26(7):1620-41.

29. Wallach T, Kramer A. Chemical chronobiology: toward drugs manipulating time. FEBS letters. 2015;589(14):1530-8.

30. Oshima T, Yamanaka I, Kumar A, Yamaguchi J, Nishiwaki-Ohkawa T, Muto K, et al. C□ H Activation Generates Period-Shortening Molecules That Target Cryptochrome in the Mammalian Circadian Clock. Angewandte Chemie International Edition. 2015;54(24):7193-7.

31. Chen Z, Yoo S-H, Takahashi JS. Small molecule modifiers of circadian clocks. Cellular and molecular life sciences. 2013;70(16):2985-98.

32. Chen Z, Yoo S-H, Park Y-S, Kim K-H, Wei S, Buhr E, et al. Identification of diverse modulators of central and peripheral circadian clocks by high-throughput chemical screening. Proceedings of the National Academy of Sciences. 2012;109(1):101-6.

33. Jang J, Chung S, Choi Y, Lim HY, Son Y, Chun SK, et al. The cryptochrome inhibitor KS15 enhances E-box-mediated transcription by disrupting the feedback action of a circadian transcription-repressor complex. Life sciences. 2018;200:49-55.

34. Lamia KA, Papp SJ, Yu RT, Barish GD, Uhlenhaut NH, Jonker JW, et al. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. Nature. 2011;480(7378):552-6.

35. Chun SK, Jang J, Chung S, Yun H, Kim N-J, Jung J-W, et al. Identification and validation of cryptochrome inhibitors that modulate the molecular circadian clock. ACS chemical biology. 2014;9(3):703-10.

36. Chun SK, Chung S, Kim H-D, Lee JH, Jang J, Kim J, et al. A synthetic cryptochrome inhibitor induces anti-proliferative effects and increases chemosensitivity in human breast cancer cells. Biochemical and biophysical research communications. 2015;467(2):441-6.

37. Isojima Y, Nakajima M, Ukai H, Fujishima H, Yamada RG, Masumoto K-h, et al. CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. Proceedings of the National Academy of Sciences. 2009;106(37):15744-9.

38. Lin H-H, Robertson KL, Bisbee HA, Farkas ME. Oncogenic and circadian effects of small molecules directly and indirectly targeting the core circadian clock. Integrative cancer therapies. 2020;19:1534735420924094.

39. Mahalingam D, Wang JS, Hamilton EP, Sarantopoulos J, Nemunaitis J, Weems G, et al. Phase 1 Open-Label, Multicenter Study of First-in-Class RORγ Agonist LYC-55716 (Cintirorgon): Safety, Tolerability, and Preliminary Evidence of Antitumor ActivityRORγ Agonist LYC-55716: Phase 1 Open-Label Multicenter Study. Clinical Cancer Research. 2019;25(12):3508-16.

40. Adeola HA, Papagerakis S, Papagerakis P. Systems biology approaches and precision oral health: a circadian clock perspective. Frontiers in physiology. 2019;10:399.

41. Janjić K, Agis H. Chronodentistry: the role & potential of molecular clocks in oral medicine. BMC Oral Health. 2019;19(1):1-12.

42. Janjić K, Kurzmann C, Moritz A, Agis H. Expression of circadian core clock genes in fibroblasts of human gingiva and periodontal ligament is modulated by L-Mimosine and hypoxia in monolayer and spheroid cultures. Archives of oral biology. 2017;79:95-9.

43. Zheng L, Seon Y, McHugh J, Papagerakis S, Papagerakis P. Clock genes show circadian rhythms in salivary glands. Journal of dental research. 2012;91(8):783-8.

44. Papagerakis S, Zheng L, Schnell S, Sartor M, Somers E, Marder W, et al. The circadian clock in oral health and diseases. Journal of dental research. 2014;93(1):27-35.

45. Zheng L, Papagerakis S, Schnell SD, Hoogerwerf WA, Papagerakis P. Expression of clock proteins in developing tooth. Gene Expression Patterns. 2011;11(3-4):202-6.

46. McLachlan JL, Smith AJ, Bujalska IJ, Cooper PR. Gene expression profiling of pulpal tissue reveals the molecular complexity of dental caries. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2005;1741(3):271-81.

47. Zieker D, Jenne I, Koenigsrainer I, Zdichavsky M, Nieselt K, Buck K, et al. Circadian expression of clock-and tumor suppressor genes in human oral mucosa. Cellular Physiology and Biochemistry. 2010;26(2):155-66.

48. Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothern RB, et al. Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. The American journal of pathology. 2001;158(5):1793-801.

49. Hsu C-M, Lin S-F, Lu C-T, Lin P-M, Yang M-Y. Altered expression of circadian clock genes in head and neck squamous cell carcinoma. Tumor Biology. 2012;33(1):149-55.

50. Wang Q, Ao Y, Yang K, Tang H, Chen D. Circadian clock gene Per2 plays an important role in cell proliferation, apoptosis and cell cycle progression in human oral squamous cell carcinoma. Oncology Reports. 2016;35(6):3387-94.

51. Zhao Q, Zheng G, Yang K, Ao Y-r, Su X-l, Li Y, Lv X-q. The clock gene PER1 plays an important role in regulating the clock gene network in human oral squamous cell carcinoma cells. Oncotarget. 2016;7(43):70290.

52. Li H-X, Fu X-J, Yang K, Chen D, Tang H, Zhao Q. The clock gene PER1 suppresses expression of tumor-related genes in human oral squamous cell carcinoma. Oncotarget. 2016;7(15):20574.

53. Fu X-J, Li H-X, Yang K, Chen D, Tang H. The important tumor suppressor role of PER1 in regulating the cyclin–cDK–cKi network in scc15 human oral squamous cell carcinoma cells. OncoTargets and therapy. 2016;9:2237.

54. Matsumoto CS, Almeida LO, Guimarães DM, Martins MD, Papagerakis P, Papagerakis S, et al. PI3K-PTEN dysregulation leads to mTOR-driven upregulation of the core clock gene BMAL1 in normal and malignant epithelial cells. Oncotarget. 2016;7(27):42393.

55. Swanson CM, Kohrt WM, Buxton OM, Everson CA, Wright Jr KP, Orwoll ES, Shea SA. The importance of the circadian system & sleep for bone health. Metabolism. 2018;84:28-43.

56. Iimura T, Nakane A, Sugiyama M, Sato H, Makino Y, Watanabe T, et al. A fluorescence spotlight on the clockwork development and metabolism of bone. Journal of bone and mineral metabolism. 2012;30:254-69.

57. Dudek M, Meng Q-J. Running on time: the role of circadian clocks in the musculoskeletal system. Biochemical journal. 2014;463(1):1-8.

58. Hirai T. Circadian clock and bone biology. Journal of Oral Biosciences. 2017;59(4):179-83.

59. Maria S, Witt-Enderby PA. Circadian regulation of bone. Circadian Rhythms and Their Impact on Aging. 2017:65-82.

60. Fujihara Y, Kondo H, Noguchi T, Togari A. Glucocorticoids mediate circadian timing in peripheral osteoclasts resulting in the circadian expression rhythm of osteoclast-related genes. Bone. 2014;61:1-9.

61. Gu X, Xing L, Shi G, Liu Z, Wang X, Qu Z, et al. The circadian mutation PER2S662G is linked to cell cycle progression and tumorigenesis. Cell Death & Differentiation. 2012;19(3):397-405.

62. Min H-Y, Kim K-M, Wee G, Kim E-J, Jang W-G. Bmal1 induces osteoblast differentiation via regulation of BMP2 expression in MC3T3-E1 cells. Life Sciences. 2016;162:41-6.

63. Samsa WE, Vasanji A, Midura RJ, Kondratov RV. Deficiency of circadian clock protein BMAL1 in mice results in a low bone mass phenotype. Bone. 2016;84:194-203.

64. Maronde E, Schilling AF, Seitz S, Schinke T, Schmutz I, Van der Horst G, et al. The clock genes Period 2 and Cryptochrome 2 differentially balance bone formation. PloS one. 2010;5(7):e11527.

65. Chapman CR, Vierck CJ. The Transition of Acute Postoperative Pain to Chronic Pain: An Integrative Overview of Research on Mechanisms. J Pain. 2017;18(4):359.e1-.e38.

66. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clinics in dermatology. 2007;25(1):9-18.

67. Segal JP, Tresidder KA, Bhatt C, Gilron I, Ghasemlou N. Circadian control of pain and neuroinflammation. Journal of Neuroscience Research. 2018;96(6):1002-20.

68. Palada V, Gilron I, Canlon B, Svensson CI, Kalso E. The circadian clock at the intercept of sleep and pain. PAIN. 2020;161(5):894-900.

69. Bruguerolle B, Labrecque G. Rhythmic pattern in pain and their chronotherapy. Advanced drug delivery reviews. 2007;59(9-10):883-95.

70. Chassard D, Bruguerolle B. Chronobiology and Anesthesia. Anesthesiology. 2004;100(2):413-27.

71. Hagenauer MH, Crodelle JA, Piltz SH, Toporikova N, Ferguson P, Booth V, editors. The modulation of pain by circadian and sleep-dependent processes: a review of the experimental evidence. Women in Mathematical Biology: Research Collaboration Workshop, NIMBioS, Knoxville, June 2015; 2017: Springer.

72. Junker U, Wirz S. Review Article: Chronobiology: influence of circadian rhythms on the therapy of severe pain. Journal of Oncology Pharmacy Practice. 2010;16(2):81-7.

73. Campagna S, Sperlinga R, Milo A, Sannuto S, Acquafredda F, Saini A, et al. The circadian rhythm of breakthrough pain episodes in terminally-ill cancer patients. Cancers. 2018;11(1):18.

74. Saini A, Tucci M, Tampellini M, Maina D, Bouraouia K, Giuliano PL, et al. Circadian variation of breakthrough pain in cancer patients. European Journal of Pain. 2013;17(2):264-70.

75. Bellamy N, Sothern RB, Campbell J. Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. The Journal of Rheumatology. 2004;31(2):379-89.

76. Bellamy N, Sothern RB, Campbell J, Buchanan WW. Circadian rhythm in pain, stiffness, and manual dexterity in rheumatoid arthritis: relation between discomfort and disability. Annals of the Rheumatic Diseases. 1991;50(4):243-8.

77. Pöllmann L. Circadian changes in the duration of local anaesthesia. Biological Rhythm Research. 1981;12(2):187-92.

78. Knezevic NN, Nader A, Pirvulescu I, Pynadath A, Rahavard BB, Candido KD. Circadian pain patterns in human pain conditions – A systematic review. Pain Practice. 2023;23(1):94-109.

79. He B, Chen Z. Molecular targets for small-molecule modulators of circadian clocks. Current drug metabolism. 2016;17(5):503-12.

80. Levi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol. 2007;47:593-628.

81. Cederroth CR, Albrecht U, Bass J, Brown SA, Dyhrfjeld-Johnsen J, Gachon F, et al. Medicine in the fourth dimension. Cell metabolism. 2019;30(2):238-50.

82. Gaspar LS, Álvaro AR, Carmo-Silva S, Mendes AF, Relógio A, Cavadas C. The importance of determining circadian parameters in pharmacological studies. British Journal of Pharmacology. 2019;176(16):2827-47.

83. Ruben MD, Smith DF, FitzGerald GA, Hogenesch JB. Dosing time matters. Science. 2019;365(6453):547-9.

84. Mure LS, Le HD, Benegiamo G, Chang MW, Rios L, Jillani N, et al. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. Science. 2018;359(6381).

85. Anafi RC, Francey LJ, Hogenesch JB, Kim J. CYCLOPS reveals human transcriptional rhythms in health and disease. Proceedings of the National Academy of Sciences. 2017;114(20):5312-7.

86. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. Proceedings of the National Academy of Sciences. 2014;111(45):16219-24.

87. Peeples L. Medicine's secret ingredient--i'? it's in the timing. Nature. 2018;556(7700):290-3.

88. Dallmann R, Okyar A, Lévi F. Dosing-time makes the poison: circadian regulation and pharmacotherapy. Trends in molecular medicine. 2016;22(5):430-45.

89. Halsas M, Hietala J, Veski P, Jürjenson H, Marvola M. Morning versus evening dosing of ibuprofen using conventional and time-controlled release formulations. International journal of pharmaceutics. 1999;189(2):179-85.

90. Reinberg A, Zagula-Mally ZW, Ghata J, Halberg F. Circadian rhythm in duration of salicylate excretion referred to phase of excretory rhythms and routine. Proceedings of the Society for Experimental Biology and Medicine. 1967;124(3):826-32.

91. Levi F, Louarn CL, Reinberg A. Timing optimizes sustained-release indomethacin treatment of osteoarthritis. Clinical Pharmacology & Therapeutics. 1985;37(1):77-84.

92. Labrecque G, Bureau J-P, Reinberg AE. Biological rhythms in the inflammatory response and in the effects of non-steroidal anti-inflammatory drugs. Pharmacology & therapeutics. 1995;66(2):285-300.

93. Al-Waeli H, Nicolau B, Stone L, Abu Nada L, Gao Q, Abdallah M, et al. Chronotherapy of non-steroidal anti-inflammatory drugs may enhance postoperative recovery. Scientific reports. 2020;10(1):468.

94. Razak PA, Richard KJ, Thankachan RP, Hafiz KA, Kumar KN, Sameer K. Geriatric oral health: a review article. Journal of international oral health: JIOH. 2014;6(6):110.

95. Huang DL, Chan KCG, Young BA. Poor oral health and quality of life in older US adults with diabetes mellitus. Journal of the American Geriatrics Society. 2013;61(10):1782-8.

96. Niesten D, van Mourik K, van der Sanden W. The impact of having natural teeth on the QoL of frail dentulous older people. A qualitative study. BMC public health. 2012;12(1):1-13.

97. Starr JM, Hall R. Predictors and correlates of edentulism in healthy older people. Current Opinion in Clinical Nutrition & Metabolic Care. 2010;13(1):19-23.

98. de Oliveira C, Watt R, Hamer M. Toothbrushing, inflammation, and risk of cardiovascular disease: results from Scottish Health Survey. Bmj. 2010;340.

99. Zheng L, Seon YJ, Mourão MA, Schnell S, Kim D, Harada H, et al. Circadian rhythms regulate amelogenesis. Bone. 2013;55(1):158-65.

100. Zheng L, Ehardt L, McAlpin B, About I, Kim D, Papagerakis S, Papagerakis P. The tick tock of odontogenesis. Experimental cell research. 2014;325(2):83-9.

101. Janjić K, Kurzmann C, Moritz A, Agis H. Core circadian clock gene expression in human dental pulp-derived cells in response to L-mimosine, hypoxia and echinomycin. European journal of oral sciences. 2018;126(4):263-71.

102. Zhao J, Zhou X, Tang Q, Yu R, Yu S, Long Y, et al. BMAL1 deficiency contributes to mandibular dysplasia by upregulating MMP3. Stem cell reports. 2018;10(1):180-95.

103. Tao J, Zhai Y, Park H, Han J, Dong J, Xie M, et al. Circadian rhythm regulates development of enamel in mouse mandibular first molar. Plos one. 2016;11(8):e0159946.

104. Evening course in dental assisting offered by the Loop College. Fortn Rev Chic Dent Soc. 1968;56(2):7-9.

105. Singla NK, Desjardins PJ, Chang PD. A comparison of the clinical and experimental characteristics of four acute surgical pain models: dental extraction, bunionectomy, joint replacement, and soft tissue surgery. PAIN®. 2014;155(3):441-56.

106. Mehlisch DR. The efficacy of combination analgesic therapy in relieving dental pain. The Journal of the American Dental Association. 2002;133(7):861-71.

107. Cei S, D'Aiuto F, Duranti E, Taddei S, Gabriele M, Ghiadoni L, Graziani F. Third molar surgical removal: a possible model of human systemic inflammation? A preliminary investigation. European Journal of Inflammation. 2012;10(1):149-52.

108. Çankaya AB, Akçay Ç, Kahraman N, Köseoğlu BG. Oral surgical procedures under local anaesthesia in day surgery. BMC Oral Health. 2018;18(1):1-4.

109. Friedman JW. The prophylactic extraction of third molars: a public health hazard. American journal of public health. 2007;97(9):1554-9.

110. Berge TI. Inability to work after surgical removal of mandibular third molars. Acta Odontologica Scandinavica. 1997;55(1):64-9.

111. Landucci A, Wosny A, Uetanabaro L, Moro A, Araujo M. Efficacy of a single dose of low-level laser therapy in reducing pain, swelling, and trismus following third molar extraction surgery. International journal of oral and maxillofacial surgery. 2016;45(3):392-8.

112. Sancho-Puchades M, Valmaseda-Castellón E, Berini-Aytés L, Gay-Escoda C. Quality of life following third molar removal under conscious sedation. Medicina oral, patologia oral y cirugia bucal. 2012;17(6):e994.

113. Moore PA, Nahouraii HS, Zovko JG, Wisniewski SR. Dental therapeutic practice patterns in the US II. Analgesics, corticosteroids, and antibiotics. General Dentistry. 2006;54(3):201-7; quiz 8, 21.

114. Moore PA, Hersh EV. Combining ibuprofen and acetaminophen for acute pain management after third-molar extractions: translating clinical research to dental practice. The Journal of the American Dental Association. 2013;144(8):898-908.

115. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Annals of internal medicine. 2018;169(7):467-73.

116. Abusamak M, Al-Tamimi M, Al-Waeli H, Tahboub K, Cai W, Morris M, et al. Chronotherapy in Dentistry: A Scoping Review. 2022.

117. Arksey H, O'Malley L. Scoping studies: towards a methodological framework. International journal of social research methodology. 2005;8(1):19-32.

118. Daudt HM, van Mossel C, Scott SJ. Enhancing the scoping study methodology: a large, inter-professional team's experience with Arksey and O'Malley's framework. BMC medical research methodology. 2013;13:1-9.

119. Colquhoun HL, Levac D, O'Brien KK, Straus S, Tricco AC, Perrier L, et al. Scoping reviews: time for clarity in definition, methods, and reporting. Journal of clinical epidemiology. 2014;67(12):1291-4.

120. Levac D, Colquhoun H, O'Brien KK. Scoping studies: advancing the methodology. Implementation science. 2010;5:1-9.

121. Tricco AC, Lillie E, Zarin W, O'brien K, Colquhoun H, Kastner M, et al. A scoping review on the conduct and reporting of scoping reviews. BMC medical research methodology. 2016;16:1-10.

122. Aromataris E, Pearson A. The systematic review: an overview. AJN The American Journal of Nursing. 2014;114(3):53-8.

123. Peters MD, Godfrey CM, McInerney P, Soares CB, Khalil H, Parker D. The Joanna Briggs Institute reviewers' manual 2015: methodology for JBI scoping reviews. 2015.

124. Davis K, Drey N, Gould D. What are scoping studies? A review of the nursing literature. International journal of nursing studies. 2009;46(10):1386-400.

125. Morris M, Boruff JT, Gore GC. Scoping reviews: establishing the role of the librarian. Journal of the Medical Library Association: JMLA. 2016;104(4):346.

126. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018;169(7):467-73.

127. Peters M, Godfrey C, McInerney P, Soares C, Khalil H, Parker D. The Joanna Briggs Institute reviewers' manual 2015: methodology for JBI scoping reviews. 2015.

128. Lockwood C, Munn Z, Porritt K. Qualitative research synthesis: methodological guidance for systematic reviewers utilizing meta-aggregation. JBI Evidence Implementation. 2015;13(3):179-87.

129. Hooijmans CR, Rovers MM, de Vries R, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC medical research methodology. 2014;14(1):1-9.

130. Ma L-L, Wang Y-Y, Yang Z-H, Huang D, Weng H, Zeng X-T. Methodological quality (risk of bias) assessment tools for primary and secondary medical studies: what are they and which is better? Military Medical Research. 2020;7(1):7.

131. Hariton E, Locascio JJ. Randomised controlled trials – the gold standard for effectiveness research. BJOG: An International Journal of Obstetrics & Gynaecology. 2018;125(13):1716-.

132. Tarnow-Mordi W, Cruz M, Morris JM, Mol BW. RCT evidence should drive clinical practice: A day without randomisation is a day without progress. BJOG: An International Journal of Obstetrics & Gynaecology. 2017;124(4):613-.

133. Andrade C. Sample Size and its Importance in Research. Indian J Psychol Med. 2020;42(1):102-3.

134. Harris JE, Raynor HA. Crossover Designs in Nutrition and Dietetics Research. J Acad Nutr Diet. 2017;117(7):1023-30.

135. Nair B. Clinical Trial Designs. Indian Dermatol Online J. 2019;10(2):193-201.

136. Cipriani A, Barbui C. What is a factorial trial? Epidemiol Psychiatr Sci. 2013;22(3):213-5.

137. Seth S, Maulik M. Randomized clinical trials design, practice and reporting. The Indian Journal of Medical Research. 2011;133(4):450-1.

138. Schulz KF, Altman DG, Moher D, Group C. WITHDRAWN: CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. Elsevier; 2010.

139. Karcioglu O, Topacoglu H, Dikme O, Dikme O. A systematic review of the pain scales in adults: Which to use? Am J Emerg Med. 2018;36(4):707-14.

140. Villafuerte-Nuñez A, Téllez-Anguiano A, Hernández-Díaz O, Rodríguez-Vera R, Gutiérrez-Gnecchi J, Salazar-Martínez J. Facial edema evaluation using digital image processing. Discrete Dynamics in Nature and Society. 2013;2013.

141. Mehra P, Reebye U, Nadershah M, Cottrell D. Efficacy of anti-inflammatory drugs in third molar surgery: a randomized clinical trial. International journal of oral and maxillofacial surgery. 2013;42(7):835-42.

142. Chander PM, Ali FM, Aher V. C-reactive protein a better indicator of inflammation after third molar extraction. Niger J Clin Pract. 2013;16(3):297-301.

143. Oberg AL, Mahoney DW. Linear mixed effects models. Topics in biostatistics. 2007:213-34.

144. MacFarland TW, Yates JM, MacFarland TW, Yates JM. Kruskal–Wallis H-test for oneway analysis of variance (ANOVA) by ranks. Introduction to nonparametric statistics for the biological sciences using R. 2016:177-211.

145. Woolson RF. Wilcoxon signed-rank test. Wiley encyclopedia of clinical trials. 2007:1-3.

146. Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV, Vail A. Meta-analyses involving cross-over trials: methodological issues. International journal of epidemiology. 2002;31(1):140-9.

147. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002;418(6901):935-41.

148. Feng G, Zhao J, Peng J, Luo B, Zhang J, Chen L, Xu Z. Circadian clock—A promising scientific target in oral science. Frontiers in Physiology. 2022:2388.

149. Cardinali DP, Brown GM, Pandi-Perumal SR. Chronotherapy. Handbook of Clinical Neurology. 2021;179:357-70.

150. Kaur G, Phillips C, Wong K, Saini B. Timing is important in medication administration: a timely review of chronotherapy research. International journal of clinical pharmacy. 2013;35(3):344-58.

151. Smolensky MH, Lemmer B, Reinberg AE. Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. Advanced drug delivery reviews. 2007;59(9-10):852-82.

152. Ballesta A, Innominato PF, Dallmann R, Rand DA, Lévi FA. Systems chronotherapeutics. Pharmacological reviews. 2017;69(2):161-99.

153. Lévi F. Circadian chronotherapy for human cancers. The lancet oncology. 2001;2(5):307-15.

154. Shuboni-Mulligan DD, Breton G, Smart D, Gilbert M, Armstrong TS. Radiation chronotherapy—clinical impact of treatment time-of-day: a systematic review. Journal of neuro-oncology. 2019;145(3):415-27.

155. Al-Waeli H, Nicolau B, Stone L, Abu Nada L, Gao Q, Abdallah MN, et al. Chronotherapy of Non-Steroidal Anti-Inflammatory Drugs May Enhance Postoperative Recovery. Scientific Reports. 2020;10(1):468.

156. Tamimi F, Abusamak M, Akkanti B, Chen Z, Yoo SH, Karmouty-Quintana H. The case for chronotherapy in Covid-19-induced acute respiratory distress syndrome. British Journal of Pharmacology. 2020;177(21):4845-50.

157. Grimshaw J. A guide to knowledge synthesis: a knowledge synthesis chapter. Ottawa: Canadian Institutes of Health Research. 2010.

158. Yang K, Zhao N, Zhao D, Chen D, Li Y. The drug efficacy and adverse reactions in a mouse model of oral squamous cell carcinoma treated with oxaliplatin at different time points during a day. Drug design, development and therapy. 2013:511-7.

159. Chen D, Cheng J, Yang K, Ma Y, Yang F. Retrospective analysis of chronomodulated chemotherapy versus conventional chemotherapy with paclitaxel, carboplatin, and 5-fluorouracil in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. OncoTargets and therapy. 2013:1507-14.

160. Zhang S, Teng T, Liao G, Liu Y, Liu Q, He W, Liu Y. Efficacy of induction chemotherapy combined with chrono-chemotherapy and intensity-modulated radiotherapy on locally advanced nasopharyngeal carcinoma. J Bu on. 2021;26:774-80.

161. Verma Y, Chauhan AK, Singh H, Sabharwal R, Bharti M, Kaur P. CHRONOMODULATED CHEMOTHERAPY AND CONCOMITANT RADIOTHERAPY, FOR THE MANAGEMENT OF LOCALLY ADVANCED, HEAD AND NECK SQUAMOUS CELL CARCINOMA. International Journal of Pharmacy & Life Sciences. 2014;5(3).

162. Zhang P, Jin F, Li Z, Wu W, Li Y, Long J, et al. A randomized phase II trial of induction chemotherapy followed by cisplatin chronotherapy versus constant rate delivery combined with radiotherapy. Chronobiology international. 2018;35(2):240-8.

163. Lin H-X, Hua Y-J, Chen Q-Y, Luo D-H, Sun R, Qiu F, et al. Randomized study of sinusoidal chronomodulated versus flat intermittent induction chemotherapy with cisplatin and 5-fluorouracil followed by traditional radiotherapy for locoregionally advanced nasopharyngeal carcinoma. Chinese journal of cancer. 2013;32(9):502.

164. Tsuchiya Y, Ushijima K, Noguchi T, Okada N, Hayasaka J-i, Jinbu Y, et al. Influence of a dosing-time on toxicities induced by docetaxel, cisplatin and 5-fluorouracil in patients with oral

squamous cell carcinoma; a cross-over pilot study. Chronobiology international. 2018;35(2):289-94.

165. Zhang Y, Chen X, Ren P, Su Z, Cao H, Zhou J, et al. Synergistic effect of combination topotecan and chronomodulated radiation therapy on xenografted human nasopharyngeal carcinoma. International Journal of Radiation Oncology* Biology* Physics. 2013;87(2):356-62.

166. Gu F, Farrugia MK, Duncan WD, Feng Y, Hutson AD, Schlecht NF, et al. Daily time of radiation treatment is associated with subsequent oral mucositis severity during radiotherapy in head and neck cancer patients. Cancer Epidemiology, Biomarkers & Prevention. 2020;29(5):949-55.

167. Kuriakose VG, Anand AS, Jayakumar K, Radhakrishnan A, Meloot S. Influence of circadian rhythm in radiation induced mucositis in head and neck malignancies. IOSR J Pharm. 2016;6:21-6.

168. Brolese EK, Cihoric N, Bojaxhiu B, Sermaxhaj B, Schanne DH, Mathier E, et al. The impact of delivery daytime and seasonality of radiotherapy for head and neck cancer on toxicity burden. Radiotherapy and oncology. 2021;158:162-6.

169. Elicin O, Brolese EK, Bojaxhiu B, Sermaxhaj B, Schanne DH, Mathier E, et al. The prognostic impact of daytime and seasonality of radiotherapy on head and neck cancer. Radiotherapy and oncology. 2021;158:293-9.

170. Goyal M, Shukla P, Gupta D, Bisht SS, Dhawan A, Gupta S, et al. Oral mucositis in morning vs. evening irradiated patients: a randomised prospective study. International journal of radiation biology. 2009;85(6):504-9.

171. Bjarnason G, MacKenzie RG, Nabid A, Hodson ID, El-Sayed S, Grimard L, et al. Comparison of toxicity associated with early morning versus late afternoon radiotherapy in

patients with head-and-neck cancer: a prospective randomized trial of the National Cancer Institute of Canada Clinical Trials Group (HN3). International Journal of Radiation Oncology* Biology* Physics. 2009;73(1):166-72.

172. Elzahi MSE, Attia SE, Elazab SH. Timing of Daily Radiotherapy for Cases of Head and Neck Cancer: Does It Make Difference? Journal of Cancer and Tumor International. 2020:12-6.

173. Igarashi K, Miyoshi K, Shinoda H, Saeki S, Mitani H. Diurnal variation in tooth movement in response to orthodontic force in rats. American journal of orthodontics and dentofacial orthopedics. 1998;114(1):8-14.

174. Miyoshi K, Igarashi K, Saeki S, Shinoda H, Mitani H. Tooth movement and changes in periodontal tissue in response to orthodontic force in rats vary depending on the time of day the force is applied. The European Journal of Orthodontics. 2001;23(4):329-38.

175. Yamada S, Saeki S, Takahashi I, Igarashi K, Shinoda H, Mitani H. Diurnal variation in the response of the mandible to orthopedic force. Journal of dental research. 2002;81(10):711-5.

176. Lemmer B, Wiemers R. Orcadian Changes in Stimulus Threshold and in the Effect of a Local Anaesthetic Drug in Human Teeth: Studies with an Electronic Pulptester. Chronobiology international. 1989;6(2):157-62.

177. Pöllmann L. Circadian changes in the duration of local anaesthesia. International journal of oral surgery. 1982;11(1):36-9.

178. Latta Jr GH. Influence of circadian periodicity on reproducibility of centric relation records for edentulous patients. The Journal of prosthetic dentistry. 1992;68(5):780-3.

179. Waghmare M, Puthenveetil SS. Chronotherapy in the treatment of oral pemphigus vulgaris: A case report. Journal of Indian Academy of Oral Medicine and Radiology. 2021;33(1):103-6.

180. Tamimi Z, Abusamak M, Al-Waeli H, Al-Tamimi M, Al Habashneh R, Ghanim M, et al. NSAID chronotherapy after impacted third molar extraction: a randomized controlled trial. Oral and Maxillofacial Surgery. 2022:1-10.

181. Restrepo DV, Sato MH, Cardoso FS, da Silva Freitas R. Should Cleft Surgery Always be Performed During the Matutine Time? Analysis of Early and Late Postoperative Complications in a Cleft Center. The Cleft Palate-Craniofacial Journal. 2020;57(11):1332-5.

182. Borgå O, Henriksson R, Bjermo H, Lilienberg E, Heldring N, Loman N. Maximum tolerated dose and pharmacokinetics of paclitaxel micellar in patients with recurrent malignant solid tumours: a dose-escalation study. Advances in Therapy. 2019;36:1150-63.

183. Bruno R, Sanderink GJ. Pharmacokinetics and metabolism of Taxotere (docetaxel). Cancer Surv. 1993;17:305-13.

184. Gouyette A, Apchin A, Foka M, Richard J-M. Pharmacokinetics of intra-arterial and intravenous cisplatin in head and neck cancer patients. European Journal of Cancer and Clinical Oncology. 1986;22(3):257-63.

185. Zeng Zl, Sun J, Guo L, Li S, Wu Mw, Qiu F, et al. Circadian rhythm in dihydropyrimidine dehydrogenase activity and reduced glutathione content in peripheral blood of nasopharyngeal carcinoma patients. Chronobiology international. 2005;22(4):741-54.

186. Elferink F, van der Vijgh WJ, Klein I, Vermorken JB, Gall HE, Pinedo HM. Pharmacokinetics of carboplatin after i.v. administration. Cancer Treat Rep. 1987;71(12):1231-7.

187. Heggie GD, Sommadossi J-P, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer research. 1987;47(8):2203-6.

188. Harris BE, Song R, Soong S-j, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer research. 1990;50(1):197-201.

189. Albert KS, Gernaat CM. Pharmacokinetics of ibuprofen. The American journal of medicine. 1984;77(1):40-6.

190. Bashar T, Apu MNH, Mostaid MS, Islam MS, Hasnat A. Pharmacokinetics and Bioavailability Study of a Prednisolone Tablet as a Single Oral Dose in Bangladeshi Healthy Volunteers. Dose Response. 2018;16(3):1559325818783932.

191. Xu J, Winkler J, Sabarinath SN, Derendorf H. Assessment of the impact of dosing time on the pharmacokinetics/pharmacodynamics of prednisolone. The AAPS journal. 2008;10:331-41.

192. Malamed SF. Handbook of local anesthesia-e-book: Elsevier health sciences; 2019.

193. Chassard D, Duflo F, de Queiroz Siqueira M, Allaouchiche B, Boselli E. Chronobiology and anaesthesia. Current Opinion in Anesthesiology. 2007;20(3):186-90.

194. Chassard D, Bruguerolle B. Chronobiology and anesthesia. ANESTHESIOLOGY-PHILADELPHIA THEN HAGERSTOWN-. 2004;100(2):413-27.

195. Bélanger P, Bruguerolle B, Labrecque G. Rhythms in pharmacokinetics: absorption, distribution, metabolism, and excretion. Physiology and pharmacology of biological rhythms: Springer; 1997. p. 177-204.

196. Pérez-González F, Abusamak M, Sáez-Alcaide LM, García-Denche JT, Marino FAT. Effect of time-dependent ibuprofen administration on the post operatory after impacted third molar extraction: a cross-over randomized controlled trial. Oral and Maxillofacial Surgery. 2022.

197. Stutzmann J. Human alveolar bone turn-over rate: a quantitative study of spontaneous and therapeutically induced variations. Malocclusion and periodontium. 1984:185-212.

198. Petrovic A, Stutzmann J, Oudet C. Turn-over of human alveolar bone removed either in the day or in the night. Biological Rhythm Research. 1981;12(2):161-6.

199. Walton JC, Bumgarner JR, Nelson RJ. Sex Differences in Circadian Rhythms. Cold Spring Harbor Perspectives in Biology. 2022:a039107.

200. Yousefzadehfard Y, Wechsler B, DeLorenzo C. Human circadian rhythm studies: Practical guidelines for inclusion/exclusion criteria and protocol. Neurobiology of Sleep and Circadian Rhythms. 2022:100080.

201. Bjarnason G, Seth A, Wang Z, Blanas N, Straume M, Martino T. Diurnal rhythms (DR) in gene expression in human oral mucosa: Implications for gender differences in toxicity, response and survival and optimal timing of targeted therapy (Rx). Journal of clinical oncology. 2007;25(18_suppl):2507-.

202. Shivanantham AH. EVALUATING THE CHEMOTHERAPEUTIC EFFECTS OF SR9009 AND ESOMEPRAZOLE ON ORAL SQUAMOUS CELL CARCINOMA USING CIRCADIAN-TAILORED INNOVATIVE THERAPEUTIC APPROACHES [Thesis]. Saskatchewan, CA: University of Saskatchewan; 2022.

203. Pountos I, Georgouli T, Blokhuis TJ, Pape HC, Giannoudis PV. Pharmacological agents and impairment of fracture healing: what is the evidence? Injury. 2008;39(4):384-94.

204. Pountos I, Georgouli T, Calori GM, Giannoudis PV. Do nonsteroidal anti-inflammatory drugs affect bone healing? A critical analysis. The Scientific World Journal. 2012;2012.

205. Mathiesen O, Wetterslev J, Kontinen V, POMMERGAARD HC, Nikolajsen L, Rosenberg J, et al. Adverse effects of perioperative paracetamol, NSAID s, glucocorticoids, gabapentinoids

and their combinations: a topical review. Acta Anaesthesiologica Scandinavica. 2014;58(10):1182-98.

206. Wester J, Haerslev T, Mathiesen F, Jespersen S, Hansen T. Naproxen versus indomethacin as night-time medication for patients with rheumatoid arthritis. Ugeskrift for laeger. 1991;153(27):1937-9.

207. Moore RA, Derry S, McQuay HJ, Wiffen PJ. Single dose oral analgesics for acute postoperative pain in adults. Cochrane Database of Systematic Reviews. 2011(9).

208. O'Connor JP, Manigrasso MB, Kim BD, Subramanian S. Fracture healing and lipid mediators. BoneKEy reports. 2014;3.

209. O'KEEFE RJ, Tiyapatanaputi P, Xie C, Li TF, Clark C, Zuscik MJ, et al. COX-2 has a critical role during incorporation of structural bone allografts. Annals of the New York Academy of Sciences. 2006;1068(1):532-42.

210. Adams SS. The propionic acids: a personal perspective. The Journal of Clinical Pharmacology. 1992;32(4):317-23.

211. Pozzi A, Gallelli L. Pain management for dentists: the role of ibuprofen. Annali di stomatologia. 2011;2(3-4 Suppl):3.

212. Seymour R, Walton J. Pain control after third molar surgery. International journal of oral surgery. 1984;13(6):457-85.

213. Seymour R, Ward-Booth P, Kelly P. Evaluation of different doses of soluble ibuprofen and ibuprofen tablets in postoperative dental pain. British journal of oral and maxillofacial surgery. 1996;34(1):110-4.

214. Derry S, Wiffen P, Moore R. Relative efficacy of oral analgesics after third molar extraction–a 2011 update. British dental journal. 2011;211(9):419-20.

215. Bergese S, Castellon-Larios K. The effectiveness of a single dose of oral ibuprofen plus caffeine in acute postoperative pain in adults. BMJ Evidence-Based Medicine. 2016;21(1):24-.

216. Foy SP, Shugars DA, Phillips C, Marciani RD, Conrad SM, White Jr RP. The impact of intravenous antibiotics on health-related quality of life outcomes and clinical recovery after third molar surgery. Journal of oral and maxillofacial surgery. 2004;62(1):15-21.

217. Deepti C, Rehan H, Mehra P. Changes in quality of life after surgical removal of impacted mandibular third molar teeth. Journal of maxillofacial and oral surgery. 2009;8(3):257-60.

218. McGrath C, Comfort M, Lo E, Luo Y. Changes in life quality following third molar surgery–the immediate postoperative period. British dental journal. 2003;194(5):265-8.

219. Al-Waeli H, Reboucas AP, Mansour A, Morris M, Tamimi F, Nicolau B. Non-steroidal anti-inflammatory drugs and bone healing in animal models—a systematic review and meta-analysis. Systematic reviews. 2021;10(1):1-20.

220. Bhandari M, Schemitsch EH. Bone formation following intramedullary femoral reaming is decreased by indomethacin and antibodies to insulin-like growth factors. Journal of orthopaedic trauma. 2002;16(10):717-22.

221. Bhattacharyya T, Levin R, Vrahas MS, Solomon DH. Nonsteroidal antiinflammatory drugs and nonunion of humeral shaft fractures. Arthritis Care & Research: Official Journal of the American College of Rheumatology. 2005;53(3):364-7.

222. Burd T, Hughes M, Anglen J. Heterotopic ossification prophylaxis with indomethacin increases the risk of long-bone nonunion. The Journal of bone and joint surgery British volume. 2003;85(5):700-5.

223. Giannoudis P, MacDonald D, Matthews S, Smith R, Furlong A, De Boer P. Nonunion of the femoral diaphysis: the influence of reaming and non-steroidal anti-inflammatory drugs. The Journal of bone and joint surgery British volume. 2000;82(5):655-8.

224. Marquez-Lara A, Hutchinson ID, Nuñez Jr F, Smith TL, Miller AN. Nonsteroidal anti-inflammatory drugs and bone-healing: a systematic review of research quality. JBJS reviews. 2016;4(3).

225. Curtis AM, Fagundes CT, Yang G, Palsson-McDermott EM, Wochal P, McGettrick AF, et al. Circadian control of innate immunity in macrophages by miR-155 targeting Bmal1. Proceedings of the National Academy of Sciences. 2015;112(23):7231-6.

226. Curtis AM, Bellet MM, Sassone-Corsi P, O'Neill LA. Circadian clock proteins and immunity. Immunity. 2014;40(2):178-86.

227. Silver AC, Arjona A, Walker WE, Fikrig E. The circadian clock controls toll-like receptor 9-mediated innate and adaptive immunity. Immunity. 2012;36(2):251-61.

228. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk H-D, et al. A circadian clock in macrophages controls inflammatory immune responses. Proceedings of the National Academy of Sciences. 2009;106(50):21407-12.

229. Hansson L, Stenström A, Thorngren K-G. Diurnal variation of longitudinal bone growth in the rabbit. Acta Orthopaedica Scandinavica. 1974;45(1-4):499-507.

230. Ivaska KK, Kakonen S-M, Gerdhem P, Obrant KJ, Pettersson K, Vaananen HK. Urinary osteocalcin as a marker of bone metabolism. Clinical chemistry. 2005;51(3):618-28.

231. Kunimoto T, Okubo N, Minami Y, Fujiwara H, Hosokawa T, Asada M, et al. A PTH-responsive circadian clock operates in ex vivo mouse femur fracture healing site. Scientific reports. 2016;6(1):1-8.

232. Iimura T, Nakane A, Sugiyama M, Sato H, Makino Y, Watanabe T, et al. A fluorescence spotlight on the clockwork development and metabolism of bone. Journal of bone and mineral metabolism. 2012;30(3):254-69.

233. Xu Y-Q, Zhang D, Jin T, Cai D-J, Wu Q, Lu Y, et al. Diurnal variation of hepatic antioxidant gene expression in mice. PloS one. 2012;7(8):e44237.

234. Na Y-R, Yoon Y-N, Son D-I, Seok S-H. Cyclooxygenase-2 inhibition blocks M2 macrophage differentiation and suppresses metastasis in murine breast cancer model. PloS one. 2013;8(5):e63451.

235. Lévi F. From circadian rhythms to cancer chronotherapeutics. Chronobiology international. 2002;19(1):1-19.

236. Lévi F, Okyar A. Circadian clocks and drug delivery systems: impact and opportunities in chronotherapeutics. Expert opinion on drug delivery. 2011;8(12):1535-41.

237. Labrecque N, Cermakian N. Circadian clocks in the immune system. Journal of biological rhythms. 2015;30(4):277-90.

238. Halberg F. Chronobiology and its promise for health care and environmental integrity. International journal of chronobiology. 1973;1(1):10-4.

239. Al-Waeli H, Nicolau B, Stone L, Abu Nada L, Gao Q, Abdallah MN, et al. Chronotherapy of Non-Steroidal Anti-Inflammatory Drugs May Enhance Postoperative Recovery. Sci Rep. 2020;10(1):468.

240. Bushra R, Aslam N. An overview of clinical pharmacology of Ibuprofen. Oman medical journal. 2010;25(3):155.

241. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. Trials. 2010;11(1):1-8.

242. Rodriguez CS. Pain measurement in the elderly: a review. Pain Management Nursing. 2001;2(2):38-46.

243. Breivik EK, Björnsson GA, Skovlund E. A comparison of pain rating scales by sampling from clinical trial data. The Clinical journal of pain. 2000;16(1):22-8.

244. Farrar JT, Young Jr JP, LaMoreaux L, Werth JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain. 2001;94(2):149-58.

245. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:14065823. 2014.

246. Geng Y, Blanco FJ, Cornelisson M, Lotz M. Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes. The Journal of Immunology. 1995;155(2):796-801.

247. Burian M, Geisslinger G. COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sites. Pharmacology & therapeutics. 2005;107(2):139-54.

248. Straatman J, Van der Peet D. C-reactive protein after major abdominal surgery: biochemical and clinical aspects. Amsterdam Medical Student Journal. 2015;2:1-3.

249. Ronchetti S, Migliorati G, Delfino D. Association of inflammatory mediators with pain perception. Biomedicine & Pharmacotherapy. 2017;96:1445-52.

250. Staff NP, Engelstad J, Klein CJ, Amrami KK, Spinner RJ, Dyck PJ, et al. Post-surgical inflammatory neuropathy. Brain. 2010;133(10):2866-80.

251. Gilron I, Bailey JM, Vandenkerkhof EG. Chronobiological characteristics of neuropathic pain: clinical predictors of diurnal pain rhythmicity. The Clinical Journal of Pain. 2013;29(9):755-9.

252. Xie S, Fan W, He H, Huang F. Role of Melatonin in the Regulation of Pain. J Pain Res. 2020;13:331-43.

253. Chang CY, Challa CK, Shah J, Eloy JD. Gabapentin in acute postoperative pain management. Biomed Res Int. 2014;2014:631756-.

254. Kim K, Brar P, Jakubowski J, Kaltman S, Lopez E. The use of corticosteroids and nonsteroidal antiinflammatory medication for the management of pain and inflammation after third molar surgery: a review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(5):630-40.

255. Baqain ZH, Karaky AA, Sawair F, Khaisat A, Duaibis R, Rajab LD. Frequency estimates and risk factors for postoperative morbidity after third molar removal: a prospective cohort study. Journal of oral and Maxillofacial Surgery. 2008;66(11):2276-83.

256. Neves FS, Souza T, Almeida S, Haiter-Neto F, Freitas D, Boscolo FN. Correlation of panoramic radiography and cone beam CT findings in the assessment of the relationship between impacted mandibular third molars and the mandibular canal. Dentomaxillofacial radiology. 2012;41(7):553-7.

257. Maglione M, Costantinides F, Bazzocchi G. Classification of impacted mandibular third molars on cone-beam CT images. Journal of clinical and experimental dentistry. 2015;7(2):e224.

258. Sağlam AA, Tüzüm MŞ. Clinical and radiologic investigation of the incidence, complications, and suitable removal times for fully impacted teeth in the Turkish population. Quintessence international. 2003;34(1).

259. Martínez-González J, Azcárate AD. Dientes retenidos. Terceros molares. Donado Cirugía bucal: Patología y técnica. 2019:199.

260. Donado Rodriguez M, Martinez-Gonzáles JM. Donado Cirugia bucal patología y técnica. España: Editorial Elsevier Masson; 2014.

261. Escoda CG, Aytés LB. Tratado de cirugía bucal: Ergon; 2015.

262. Deshpande P, V. Guledgud M, Patil K. Proximity of impacted mandibular third molars to the inferior alveolar canal and its radiographic predictors: a panoramic radiographic study. Journal of maxillofacial and oral surgery. 2013;12:145-51.

263. Tulloch JC, Antczak-Bouckoms AA, Ung N. Evaluation of the costs and relative effectiveness of alternative strategies for the removal of mandibular third molars. International journal of technology assessment in health care. 1990;6(4):505-15.

264. Gatchel RJ, Okifuji A. Evidence-based scientific data documenting the treatment and cost-effectiveness of comprehensive pain programs for chronic nonmalignant pain. The Journal of Pain. 2006;7(11):779-93.

265. Moore RA, Derry S, Aldington D, Wiffen PJ. Single dose oral analgesics for acute postoperative pain in adults-an overview of Cochrane reviews. Cochrane Database of Systematic Reviews. 2015(9).

266. Gómez-Acebo I, Dierssen-Sotos T, De Pedro M, Pérez-Gómez B, Castaño-Vinyals G, Fernández-Villa T, et al. Epidemiology of non-steroidal anti-inflammatory drugs consumption in Spain. The MCC-Spain study. BMC Public Health. 2018;18:1-13.

267. Acheson RM, Chan Y-K, Payne M. New Haven survey of joint diseases. Journal of Chronic Diseases. 1969;21(8):533-42.

268. Ingpen M. A controlled clinical trial of sustained-action dextropropoxyphene hydrochloride. The British journal of clinical practice. 1969;23(3):113-5.

269. Kunimoto T, Okubo N, Minami Y, Fujiwara H, Hosokawa T, Asada M, et al. A PTH-responsive circadian clock operates in ex vivo mouse femur fracture healing site. Scientific reports. 2016;6(1):22409.

270. Scheiermann C, Kunisaki Y, Frenette PS. Circadian control of the immune system. Nature Reviews Immunology. 2013;13(3):190-8.

271. Brea M, Micó JA. TNF and cytokines and pain: Beyond the tisular inflammation. Reumatologia Clinica. 2009;5:1-4.

272. Lemmer B. Chronobiology, drug-delivery, and chronotherapeutics. Advanced drug delivery reviews. 2007;59(9-10):825-7.

273. Kakan X, Chen P, Zhang J. Clock gene mPer2 functions in diurnal variation of acetaminophen induced hepatotoxicity in mice. Experimental and toxicologic pathology. 2011;63(6):581-5.

274. Xu Y-Q, Zhang D, Jin T, Cai D-J, Wu Q, Lu Y, et al. Diurnal variation of hepatic antioxidant gene expression in mice. 2012.

275. Dwan K, Li T, Altman DG, Elbourne D. CONSORT 2010 statement: extension to randomised crossover trials. bmj. 2019;366.

276. Janjua OS, Baig Z, Manzoor A, Abbas T. Accuracy of Pederson and modified Parant scale for predicting difficulty level of mandibular third molars. Arch Orofac Sci. 2013;8(1):9-13.

277. Amin MM, Laskin DM. Prophylactic use of indomethacin for prevention of postsurgical complications after removal of impacted third molars. Oral surgery, oral medicine, oral pathology. 1983;55(5):448-51.

278. Rietveld W. Chronobiology. Hormone Research in Paediatrics. 1990;33(2-4):53-7.

279. Smolensky MH, D'alonzo GE. Medical chronobiology: concepts and applications. American Review of Respiratory Disease. 1993;147:S2-S.

280. Stranges PM, Drew AM, Rafferty P, Shuster JE, Brooks AD. Treatment of hypertension with chronotherapy: is it time of drug administration? Annals of Pharmacotherapy. 2015;49(3):323-34.

281. Bowles N, Thosar S, Herzig M, Shea S. Chronotherapy for hypertension. Current hypertension reports. 2018;20:1-24.

282. Shuboni-Mulligan DD, Breton G, Smart D, Gilbert M, Armstrong TS. Radiation chronotherapy—clinical impact of treatment time-of-day: A systematic review. Journal of neuro-oncology. 2019;145:415-27.

283. Song J-U, Park HK, Lee J. Impact of dosage timing of once-daily inhaled corticosteroids in asthma: a systematic review and meta-analysis. Annals of Allergy, Asthma & Immunology. 2018;120(5):512-9.

284. Pergolizzi JV, Magnusson P, LeQuang JA, Gharibo C, Varrassi G. The pharmacological management of dental pain. Expert opinion on pharmacotherapy. 2020;21(5):591-601.

285. Sáez-Alcaide LM, Molinero-Mourelle P, González-Serrano J, Rubio-Alonso L, Bornstein MM, López-Quiles J. Efficacy of a topical gel containing chitosan, chlorhexidine, allantoin and dexpanthenol for pain and inflammation control after third molar surgery: A randomized and placebo-controlled clinical trial. Medicina oral, patologia oral y cirugia bucal. 2020;25(5):e644.

286. do Nascimento-Junior EM, Dos Santos GMS, Mendes MLT, Cenci M, Correa MB, Pereira-Cenci T, Martins-Filho PRS. Cryotherapy in reducing pain, trismus, and facial swelling after third-molar surgery: systematic review and meta-analysis of randomized clinical trials. The Journal of the American Dental Association. 2019;150(4):269-77. e1.

287. Isola G, Matarese M, Ramaglia L, Iorio-Siciliano V, Cordasco G, Matarese G. Efficacy of a drug composed of herbal extracts on postoperative discomfort after surgical removal of impacted mandibular third molar: A randomized, triple-blind, controlled clinical trial. Clinical Oral Investigations. 2019;23:2443-53.

288. Kimura G. Pathogenesis of edema and its classification. Nihon rinsho Japanese journal of clinical medicine. 2005;63(1):11-6.

289. Desjardins P, Alvarado F, Gil M, González M, Guajardo R. Efficacy and safety of two fixed-dose combinations of tramadol hydrochloride and diclofenac sodium in postoperative dental pain. Pain Medicine. 2020;21(10):2447-57.

290. Daniels SE, Atkinson HC, Stanescu I, Frampton C. Analgesic efficacy of an acetaminophen/ibuprofen fixed-dose combination in moderate to severe postoperative dental pain: a randomized, double-blind, parallel-group, placebo-controlled trial. Clinical therapeutics. 2018;40(10):1765-76. e5.

291. Food, Administration D. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products—general considerations. Food and Drug Administration, Washington, DC. 2003.

292. Printezi MI, Kilgallen AB, Bond MJ, Štibler U, Putker M, Teske AJ, et al. Toxicity and efficacy of chronomodulated chemotherapy: a systematic review. The Lancet Oncology. 2022;23(3):e129-e43.

293. Deng S, Qi M, Gong P, Tan Z. The circadian clock component BMAL1 regulates osteogenesis in osseointegration. Frontiers in Pediatrics. 2022;10:1091296.

294. Okawa H, Egusa H, Nishimura I. Implications of the circadian clock in implant dentistry. Dental materials journal. 2020;39(2):173-80.

295. Škrlec I. The influence of dental implants on the circadian clock and the role of melatonin in the oral cavity. Exploratory research and hypothesis in medicine. 2023;8(2):143-9.

296. Hallare J, Gerriets V. Half Life. 2020.

297. Barclay-Goddard R, Epstein JD, Mayo NE. Response shift: a brief overview and proposed research priorities. Quality of Life Research. 2009;18(3):335-46.

12 Chapter 12: Appendix I – Supplementary materials of manuscript I

Supplemental Table 1. Search strategy for each database.

MEDLINE(Ovid)

Steps

1. exp Circadian Rhythm/

2. exp Circadian Clocks/

3. exp Biological Clocks/

4. exp Chronotherapy/

5. ((circadian or biological or diurnal or nyctohemeral or twenty-four hour or 24-hour or ultradian) adj1 (rhythm? or clock?)).tw,kf.

6. chronotherapy.tw,kf.

7. (morning? or afternoon? or evening?).tw,kf.

8. (time? of day or morning? or afternoon? or evening?).tw,kf.

9. or/1-8

10. exp Dentists/ or exp Dentistry/ or exp Dental Clinics/ or exp Dental Auxiliaries/

11. dentist* or denturist* or ((dental or oral) adj3 (health or care or surgeon? or office? or clinic? or assistant? or nurse? or hygien* or practitioner? or professional? or auxiliar*))).tw,kf.

12. exp Dental Health Services/

13. exp Stomatognathic Diseases/

14. exp Stomatognathic System/

15. exp Oral Health/

16. (((periodontal or periapical or stomatognathic or oral or mouth or dental) adj3 disease?) or periodontitis or pericoronitis or peri-implantitis or caries or ((tooth or teeth) adj3 (loss or mobility)) or (gingival adj3 (h?emorrhage? or overgrowth or recession)) or gingivitis or (oral adj1 (hygiene or health))).tw,kf.

17. (intra-oral* or intraoral* or buccal or gingiv* or gum? or palat* or lingual* or mandib* or maxill* or glossal* or otor?inolaryngolog* or larynx* or laryngeal* or sinus or hypopharynx or hypopharyngeal* or nasopharynx or nasopharyngeal* or oropharynx or oropharyngeal or tonsil* or trachea* or cheek* or pharynx or pharyngeal or retromolar or alveolar or tonsil* or sinonasal or sinus* or vestib* or piriform or post-cricoid or glottic or subglottic or superglottic or transglottic or "unknown primary" or trigone or maxillofacial*).tw,kf.

18. or/10-17

19. 9 and 18

EMBASE

Steps

1. exp biological rhythm/

2. exp chronotherapy/

3. ((circadian or biological or diurnal or nyctohemeral or twenty-four hour or 24-hour or ultradian) adj1 (rhythm? or clock?)).tw,kf.

4. chronotherapy.tw,kf.

5. (morning? or afternoon? or evening?).tw,kf.

6. (time? of day or morning? or afternoon? or evening?).tw,kf.

7. or/1-6

8. exp dentist/ or exp dentistry/ or exp dental clinic/ or exp dental auxiliary/

9.(dentist* or denturist* or ((dental or oral) adj3 (health or care or surgeon? or office? or clinic? or assistant? or nurse? or hygien* or practitioner? or professional? or auxiliar*))).tw,kf.

10. exp dental clinic/

12. exp mouth disease/

12. exp stomatognathic system/

13. (((periodontal or periapical or stomatognathic or oral or mouth or dental) adj3 disease?) or periodontitis or pericoronitis or peri-implantitis or caries or ((tooth or teeth) adj3 (loss or mobility)) or (gingival adj3 (h?emorrhage? or overgrowth or recession)) or gingivitis or (oral adj1 (hygiene or health))).tw,kf.

14. (intra-oral* or intraoral* or buccal or gingiv* or gum? or palat* or lingual* or mandib* or maxill* or glossal* or otor?inolaryngolog* or larynx* or laryngeal* or sinus or hypopharynx or hypopharyngeal* or nasopharynx or nasopharyngeal* or oropharynx or oropharyngeal or tonsil* or trachea* or cheek* or pharynx or pharyngeal or retromolar or alveolar or tonsil* or sinonasal or sinus* or vestib* or piriform or post-cricoid or glottic or subglottic or superglottic or transglottic or "unknown primary" or trigone or maxillofacial*).tw,kf.

15. or/8-14

16. 7 and 15

CINAHL

Steps

S1. (MH "Circadian Rhythm+")

S2. (MH "Biological Clocks+")

S3. (MH "Cronotherapy+")

S4. TI ((circadian or biological or diurnal or nyctohemeral or twenty-four hour or 24-hour or ultradian) N1 (rhythm# or clock#)) OR AB ((circadian or biological or diurnal or nyctohemeral or twenty-four hour or 24-hour or ultradian) N1 (rhythm# or clock#))

S5. TI (time# of day OR morning# or afternoon# or evening#) OR AB (time# of day OR morning# or afternoon# or evening#)

S6. S1 OR S2 OR S3 OR S4 OR S5

S7. (MH "Dentists+") or (MH "Dentistry+") or (MH "Dental Clinics+") or (MH "Dental Auxiliaries+")

S8. TI (dentist* or denturist* or ((dental or oral) N3 (health or care or surgeon# or office# or clinic# or assistant# or nurse# or hygien* or practitioner# or professional# or auxiliar*))) OR AB (dentist* or denturist* or ((dental or oral) N3 (health or care or surgeon# or office# or clinic# or assistant# or nurse# or hygien* or practitioner# or professional# or auxiliar*)))

S9. (MH "Dental Health Services+")

S10. (MH "Stomatognathic Diseases+")

S11. (MH "Oral Health+")

S12. (((periodontal or periapical or stomatognathic or oral or mouth or dental) N3 disease#) or periodontitis or pericoronitis or peri-implantitis or caries or ((tooth or teeth) N3 (loss or mobility)) or (gingival N3 (h#emorrhage# or overgrowth or recession)) or gingivitis or (oral N1 (hygiene or health))) OR AB (((periodontal or periapical or stomatognathic or oral or mouth or dental) N3 disease#) or periodontitis or pericoronitis or peri-implantitis or caries or

((tooth or teeth) N3 (loss or mobility)) or (gingival N3 (h#emorrhage# or overgrowth or recession)) or gingivitis or (oral N1 (hygiene or health)))

S13. TI (intra-oral* or intraoral* or buccal or gingiv* or gum# or palat* or lingual* or mandib* or maxill* or glossal* or otor#inolaryngolog* or larynx* or laryngeal* or sinus or hypopharynx or hypopharyngeal* or nasopharynx or nasopharyngeal* or oropharynx or oropharyngeal or tonsil* or trachea* or cheek* or pharynx or pharyngeal or retromolar or alveolar or tonsil* or sinonasal or sinus* or vestib* or piriform or post-cricoid or glottic or subglottic or superglottic or transglottic or "unknown primary" or trigone or maxillofacial*) OR AB (intra-oral* or intraoral* or buccal or gingiv* or gum# or palat* or lingual* or mandib* or maxill* or glossal* or otor#inolaryngolog* or larynx* or laryngeal* or sinus or hypopharynx or hypopharyngeal* or nasopharynx or nasopharyngeal* or oropharynx or oropharyngeal or tonsil* or trachea* or cheek* or pharynx or pharyngeal or retromolar or alveolar or tonsil* or sinonasal or sinus* or vestib* or piriform or post-cricoid or glottic or subglottic or superglottic or transglottic or "unknown primary" or trigone or maxillofacial*)

S14. S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13

S15. S6 AND S14

SCOPUS

(INDEXTERMS (circadian OR biological AND clock OR chronotherapy) OR TITLE-ABS-KEY-AUTH ((circadian OR biological OR diurnal OR nyctohemeral OR "twenty-four hour" OR 24-hour OR ultradian) W/1 (rhythm* OR clock*)) OR TITLE-ABS-KEY-AUTH (chronotherapy OR "time* of day" OR morning* OR afternoon* OR evening*)) AND ((INDEXTERMS (dentist* OR dental) OR TITLE-ABS-KEY-AUTH

(dentist* OR denturist* OR ((dental OR oral) W/3 (health OR care OR surgeon* OR office* OR clinic* OR assistant* OR nurse? OR hygien* OR practitioner* OR professional* OR auxiliar*)))) OR (TITLE-ABS-KEY-AUTH (((periodontal OR periapical OR stomatognathic OR oral OR mouth OR dental) W/3 disease*) OR periodontitis OR pericoronitis OR peri-implantitis OR caries OR ((tooth OR teeth) W/3 (loss OR mobility)) OR (gingival W/3 (hemorrhage* OR haemorrhage* OR overgrowth OR recession)) OR gingivitis OR (oral W/1 (hygiene OR health)))) OR (INDEXTERMS (intra-oral* OR intraoral* OR buccal OR gingiv* OR gum OR gums OR palat* OR lingual* OR mandib* OR maxill* OR glossal* OR otorinolaryngolog* OR otorhinolaryngolog OR larynx* OR laryngeal* OR sinus OR hypopharynx OR hypopharyngeal* OR nasopharynx OR nasopharyngeal* OR oropharynx OR oropharyngeal OR tonsil* OR trachea* OR cheek* OR pharynx OR pharyngeal OR retromolar OR alveolar OR tonsil* OR sinonasal OR sinus* OR vestib* OR piriform OR post-cricoid OR glottic OR subglottic OR superglottic OR transglottic OR "unknown primary" OR trigone OR maxillofacial*))) AND NOT DBCOLL(medl)

Supplemental Table 2. Detailed overview of included studies discussing chronotherapy applications in dentistry

First Author/Year	Comparator Groups	Endpoints	Best time for intervention
Yang et al. 2013	Oxiplatin injection at 4,10,16 and 22 HALO and Control	Therapeutic efficacy, adverse effects, and survival time	16 and 22 HALO
Chen D et al. 2013	Intervention: Paclitaxel (03:00h – 05:00h) and Carboplatin (16:00h – 20:00h) on Day 1, while 5-Fu on Day 1 to 5 at 22:00h – 07:00h. Control: started at 09:00h – 11:00h and finished before 17:30h.	Tumor objective response rate, overall survival, progression-free survival, and adverse events incidence.	Paclitaxel (03:00h – 05:00h), Carboplatin (16:00h – 20:00h) and 5-Fu (22:00h – 07:00h)
Zhang S et al. 2021	After induction chemotherapy using docetaxel + cisplatin + 5-fluorouracil (5-FU) (TPF) regimen and during radiotherapy, Chronochetherapy group: Cisplatin between 10:00h and 22:00h with peak delivery at 16:00h. Control: conventional intravenous instilling of Cisplatin (10:00h to 22:00h)	WHO criteria for evaluating the efficacy of solid tumors, Common Terminology Criteria for Adverse Events (CT- CAE 3. 0) and peripheral blood immune cells.	Cisplatin between 10:00h and 22:00h with peak delivery at 16:00h

Verma et al. 2014	Group 1: Cisplatin at 06:00h. Group 2: Cisplatin at 18:00h.	Response to treatment and (RTOG) toxicity criteria	Cisplatin at 18:00h
Zhang P et al. 2017	Intervention: Cisplatin chronomodulated infusion (10:00h – 22:00h with peak delivery at 16:00h) Control: flat intermittent infusion (10:00h – 14:00h)	Adverse reactions, effect on immune functions, and therapeutic efficacy	Cisplatin chronomodulated infusion (10:00h – 22:00h with peak delivery at 16:00h)
Lin et al. 2013	Cisplatin and 5-Fu chronomodulated infusion (Cisplatin 10:00h – 22:00h with peak delivery of at 16:00h, and 5-Fu 22:00h -10:00h with a peak delivery at 04:00h) vs constant flat infusion.	Chemotherapeutic acute toxicity and response and overall survival	Cisplatin 10:00h – 22:00h with peak delivery of at 16:00h and 5-Fu 22:00h - 10:00h with a peak delivery at 04:00h
Tsuchiya et al. 2018	Docetaxel 60mg/m2 1-hour infusion, Cisplatin 60mg/m2 2-hour infusion, 5-Fu 600mg/m2 24-hour infusion: Morning dose group (10:30h) and Evening dose group (17:00h)	Drug-induced toxicity and adverse effects	Docetaxel, Cisplatin, and 5-Fu at 17:00h

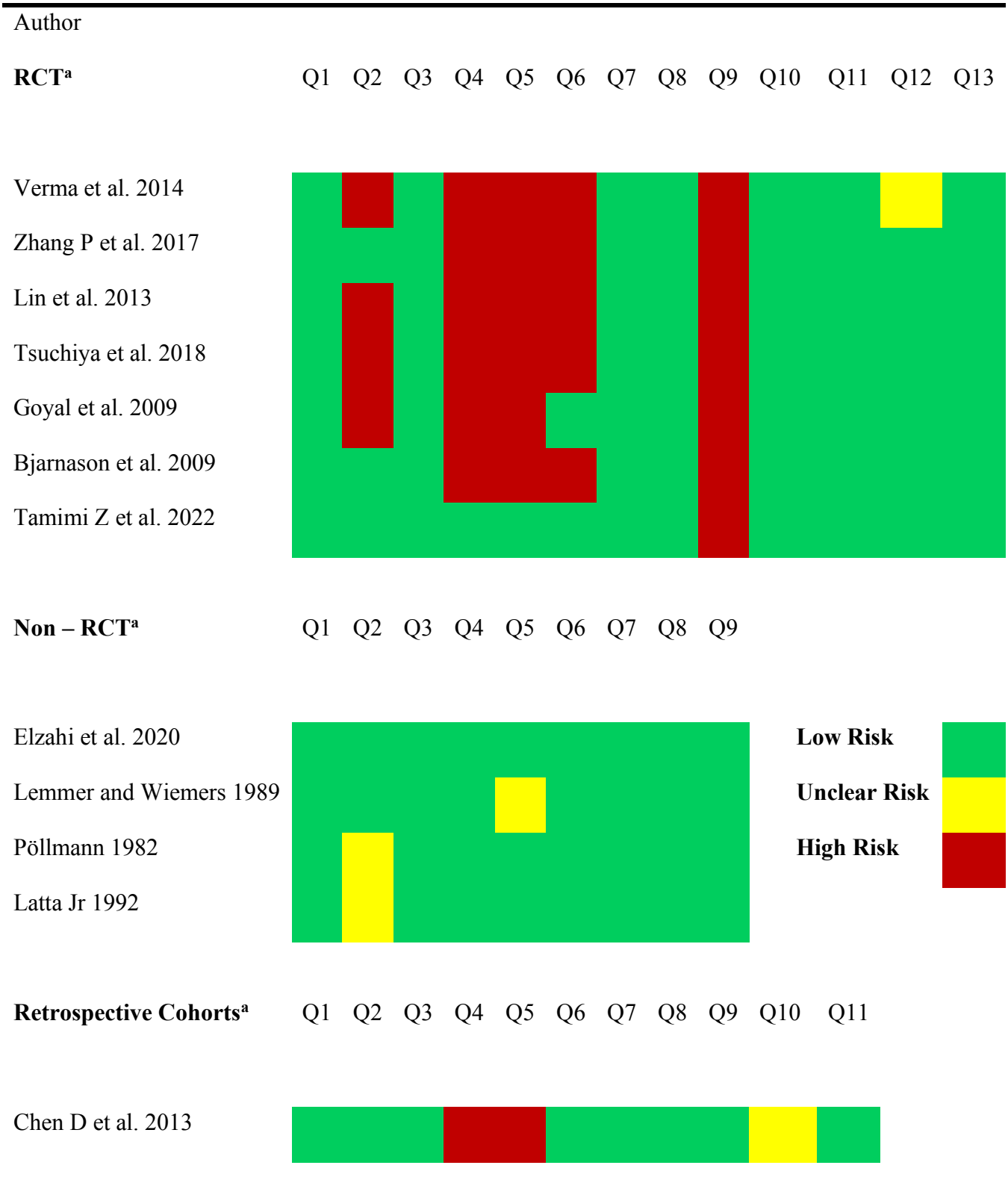
Zhang Y et al. 2013	Time-points (3,9,15,21 HALO), Treatment modalities (TPT, RT, TPT+RT), Irradiation dose (10,15,20 Gy) + Control	Radiosensitization effect and therapeutic efficacy of TPT with RT	TPT+RT at 15 HALO
Gu et al. 2020	7 groups: (1 =08:30h–09:30h; 2 = 09:30h–10:30h; 3 = 10:30h–11:30h; 4 = 11:30h–12:30h; 5=12:30h–14:00h; 6 = 14:00h–15:00h; 7 =15:00h–16:30h)	Mouth Throat Soreness (MTS)	RT at 08:30h – 09:30h
Kuriakose et al. 2016	RT in morning (08:00h -11:00h) and evening (17:00h - 20:00h)	Radiation Therapy Oncology Group (RTOG) criteria	RT at 08:00h – 11:00h
Brolese et al. 2021	RT in AM and PM (dichotomized by noon (12:00h)) and RT in DARK and LIGHT (Sept – March and March – Sept, retrospectivity)	Common Terminology Criteria for Adverse Events (CTCAE)	RT from March to September
Elicin et al. 2021	RT in AM and PM (dichotomized by noon (12:00h)) and RT in DARK and LIGHT (Sept – March and March – Sept, retrospectivity)	loco-regional control (LRC), progression-free (PFS) and overall survival (OS).	RT from September to March
Goyal et al. 2009	RT in morning (08:00h–11:00h) and evening (15:00h–18:00h)	Radiation Therapy Oncology Group (RTOG) criteria	RT at 08:00h – 11:00h

Bjarnason et al. 2009	RT in morning (08:00h -10:00h) and afternoon (16:00h - 18:00h)	Radiation Therapy Oncology Group (RTOG) criteria	RT at 08:00h – 10:00h
Elzahi et al. 2020	RT in morning (06:00h -08:00h) and afternoon (13:00h - 15:00h)	Soreness quality scores	RT 06:00h – 08:00h
Igarashi et al. 1998	Orthodontic expansive force application on upper first molar (Whole day + light (07:00h-19:00h) +Dark (19:00h-07:00h) + Control)	Tooth movement, bone formation and resorption	Light period (07:00h-19:00h)
Miyoshi et al. 2001	Orthodontic expansive force application on upper first molar (Whole day + light (07:00h-19:00h) +Dark (19:00h-07:00h) + Control)	Tooth movement, bone formation and resorption and PDL hyalinization	Light period (07:00h-19:00h)
Yamada et al. 2002	Mandibular retractive force application on the mandible condyle (Whole day + light (08:00h-20:00h) +Dark (20:00h-08:00h) + Control)	Inhibition of condylar growth and differentiation and proliferation of chondrocytes	Light period (08:00h-20:00h)
Lemmer and Wiemers 1989	Stimulus threshold using the electronic pulp tester DP2000 in 28 healthy patients.	Stimulus threshold to quantify drug effect by time to reach peak effect, duration at peak effect,	Local anesthesia at 14:00h & 17:00h

	Local anesthesia (0.8 ml Articaine with epinephrine) at one of the following times (08:00h, 11:00h, 14:00h and 17:00h local time) in 36 patients. Local anesthesia (0.4 ml Articaine with epinephrine) at one of the following times (14:00h and 17:00h) in 19 patients.	time to return to baseline threshold	
Pöllmann 1982	Different time (06:00h – 23:00h, 1 group per hour) of LA injections after surgeries	Numbness duration and pain onset after surgery	Local anesthesia at 14:00h-15:00h
Latta Jr 1992	Centric relation records for CD; Twice remounted in the morning (AM) + twice remounted in the afternoon (PM) + remounted once in the morning (AM) and once in the afternoon (PM)	Positional changes in centric relation records for edentulous patients	Centric records measured at 12:00h
Waghmare and Puthenveetil 2021	First regimen: patient received Prednisolone 40mg BID. Second regimen: Prednisolone 60mg at 06:00h.	Complete remission	Prednisolone at 06:00h.
Tamimi Z et al. 2022	Chronotherapy: Ibuprofen 400 mg at 0:800h, 13:00h and a placebo at 20:00h. Control: Ibuprofen 400 mg at 08:00h, 13:00h and 20:00h.	Post-Operative Pain: Visual Analogue Scale	Daytime administration of Ibuprofen (08:00h & 13:00h)

		Post-Operative Healing Indicators: facial swelling, mouth opening, and CRP.	
Restrepo et al 2020	CL, CLP and CP surgeries in morning (07:00h – 13:00h) and evening (13:00h-19:00h)	Postoperative complications (immediate and late)	N/A

Supplemental Table 3. Risk of bias assessment for included studies



Zhang S et al. 2021

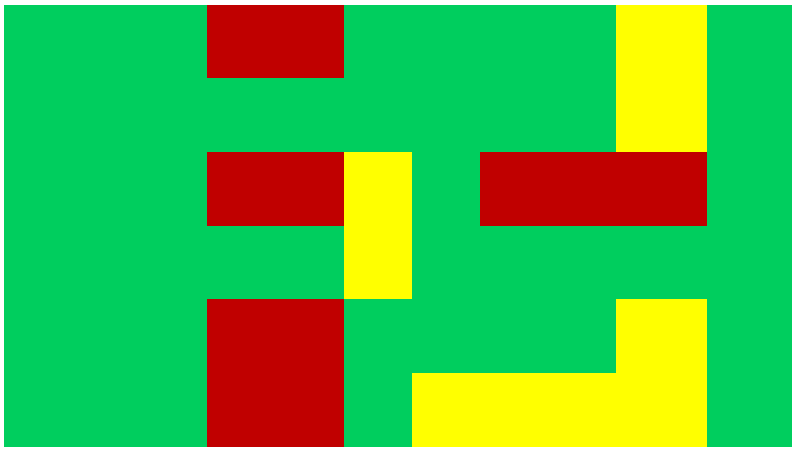
Gu et al. 2020

Kuriakose et al. 2016

Brolese et al. 2021

Elicin et al. 2021

Restrepo et al 2020



Case Reports[a] Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8

Waghmare and

Puthenveetil 2021

Animal Studies[b] Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 Q9 Q10 Q11

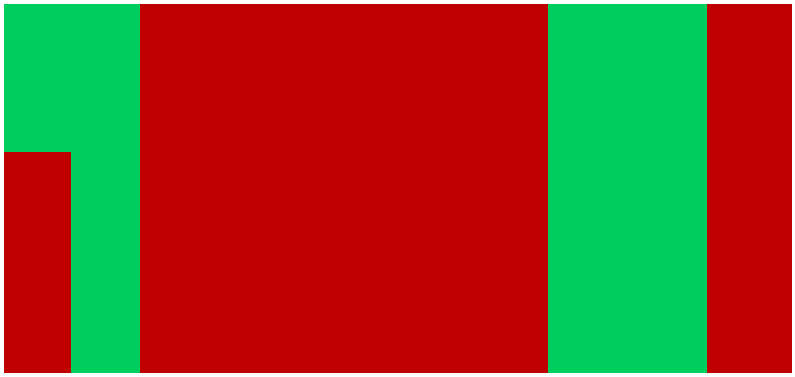
Yang et al. 2013

Zhang Y et al. 2013

Igarashi et al. 1998

Miyoshi et al. 2001

Yamada et al. 2002



Abbreviations: RCT: Randomized Controlled Trials; Q: Question. [a]: Joanna Briggs Institute (JBI) risk of bias assessment tool; [b]: SYstematic Review Center for Laboratory animal Experimentation's

(SYRCLE) risk of bias assessment tool. See supplemental document, pages 6 & 7, for assessment questions.

Joanna Briggs Institute risk of bias assessment questions for RCT:

Q1. Was true randomization used for assignment of participants to treatment groups?

Q2. Was allocation to treatment groups concealed?

Q3. Were treatment groups similar at the baseline?

Q4. Were participants blind to treatment assignment?

Q5. Were those delivering treatment blind to treatment assignment?

Q6. Were outcomes assessors blind to treatment assignment?

Q7. Were treatment groups treated identically other than the intervention of interest?

Q8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?

Q9. Were participants analyzed in the groups to which they were randomized?

Q10. Were outcomes measured in the same way for treatment groups?

Q11. Were outcomes measured in a reliable way?

Q12. Was appropriate statistical analysis used?

Q13. Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?

Joanna Briggs Institute risk of bias assessment questions for non-RCT:

Q1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?

Q2. Were the participants included in any comparisons similar?

Q3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?

Q4. Was there a control group?

Q5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?

Q6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?

Q7. Were the outcomes of participants included in any comparisons measured in the same way?

Q8. Were outcomes measured in a reliable way?

Q9. Was appropriate statistical analysis used?

Joanna Briggs Institute risk of bias assessment questions for Cohorts:

Q1. Were the two groups similar and recruited from the same population?

Q2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?

Q3. Was the exposure measured in a valid and reliable way?

Q4. Were confounding factors identified?

Q5. Were strategies to deal with confounding factors stated?

Q6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?

Q7. Were the outcomes measured in a valid and reliable way?

Q8. Was the follow up time reported and sufficient to be long enough for outcomes to occur?

Q9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?

Q10. Were strategies to address incomplete follow up utilized?

Q11. Was appropriate statistical analysis used?

Joanna Briggs Institute risk of bias assessment questions for Case Reports:

Q1. Were patient's demographic characteristics clearly described?

Q2. Was the patient's history clearly described and presented as a timeline?

Q3. Was the current clinical condition of the patient on presentation clearly described?

Q4. Were diagnostic tests or assessment methods and the results clearly described?

Q5. Was the intervention(s) or treatment procedure(s) clearly described?

Q6. Was the post-intervention clinical condition clearly described?

Q7. Were adverse events (harms) or unanticipated events identified and described?

Q8. Does the case report provide takeaway lessons?

SYstematic Review Center for Laboratory animal Experimentation's (SYRCLE) risk of bias assessment questions for animal studies:

Q1. Was the allocation sequence adequately generated and applied?

Q2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?

Q3. Was allocation adequately concealed?

Q4. Were the animals randomly housed during the experiment?

Q5. Were the caregivers and /or investigators blinded from knowledge which intervention each animal received during the experiment?

Q6. Were animals selected at random for outcome assessment?

Q7. Was the outcome assessor blinded?

Q8. Were incomplete outcome data adequately addressed?

Q9. Are reports of the study free of suggestion of selective reporting?

Q10. Was the study apparently free of other problems that could put it at high risk of bias?

Q11. Was there a sample size calculation?

Supplemental Table 4. Included studies that reported sub-group analyses

Reference	Sub-group analysis	Results
Bjarnason et al. 2009	Smokers vs Non-smokers	Significant reduction on grade 3 or greater mucositis in morning RT vs evening RT
	Irradiation doses and fractions	Significant reduction on grade 3 or greater mucositis in morning RT vs evening RT treated with a dose of 66–70 Gy in 33–35 fractions
	Males vs Females	Incidence of grade 3 or greater mucositis in Morning RT vs Evening RT Males: 49.4% vs 64.1, p= 0.078; Females:65.2% vs 56.6%, p=0.76
Verma et al. 2014	TNM staging system	Non-significant differences between various T and N stages.
Kuriakose et al. 2016	Smoking vs non smoker	Significantly higher severity of oral mucositis in smokers vs non-smokers
	Concurrent Chemotherapy	Significantly higher severity of oral mucositis with concurrent chemotherapy vs no concurrent chemotherapy
Lemmer & Wiemers 1988	Chronotype (Horne-Ostberg Questionnaire)	No significant difference.

13 Chapter 13: Appendix II – Supplementary materials of manuscript II

Supplemental Table 5. Rescue medication consumption postoperatively.

	Control	Treatment	p-Value
n	37	33	
RM* Day 1 *(%)*			
Yes	9 (24.3)	13 (39.4)	0.272 [a]
No	28 (75.7)	20 (60.6)	
Total RM Pills Day 1 *(median [IQR])*	0.00 [0.00, 0.00]	0.00 [0.00, 1.00]	0.243 [b]
RM Day 2 *(%)*			
Yes	10 (27.0)	15 (45.5)	0.175 [a]
No	27 (73.0)	18 (54.5)	
Total RM Pills Day 2 *(median [IQR])*	0.00 [0.00, 1.00]	0.00 [0.00, 2.00]	0.123 [b]
RM Day 3 *(%)*			
Yes	9 (24.3)	12 (36.4)	0.403 [a]
No	28 (75.7)	21 (63.6)	
Total RM Pills Day 3 *(median [IQR])*	0.00 [0.00, 0.00]	0.00 [0.00, 1.00]	0.403 [b]
RM Day 4 *(%)*			
Yes	8 (21.6)	10 (30.3)	0.375 [a]
No	29 (78.4)	22 (66.7)	
Missing	0 (0.0)	1 (3.0)	
Total RM Pills Day 4 *(median [IQR])*	0.00 [0.00, 0.00]	0.00 [0.00, 1.00]	0.369 [b]

[a] *Pearson's Chi-squared test,* [b] *Kruskal-Wallis Rank Sum Test.*

* *Rescue Medication*

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3&4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	4

Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5&6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	5
	11b	If relevant, description of the similarity of interventions	5

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	6&7
	13b	For each group, losses and exclusions after randomisation, together with reasons	6&7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Table 2
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Table 1

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	8
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	7&8
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	7&8
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	8