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To investigate the effect of plasma rich in growth factors (PRGF) on healing and quality of life following mandibular third molar removal

Thesis presented by
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for the degree of
Doctor of Clinical Dentistry (Oral Surgery)

University College Cork
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2021

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TERMS AND ABBREVIATIONS

AAOMS	American association of oral and maxillofacial surgeons
AFA	Autologous fibrin adhesive
AGP	Aerosol-generating procedure
AO	Alveolar osteitis
APC	Autologous platelet concentrate
A-PRF	Advanced platelet-rich fibrin
BAOMS	British association of oral and maxillofacial surgeons
BC	Buffy coat
CBCT	Cone beam computed tomography
COSMIN	Consensus-based Standards for the selection of health Measurement INstruments
cPRP	Concentrated platelet-rich plasma
COMET	Core Outcome Measures in Effectiveness Trials
COS	Core outcome sets
COS-STAD	Core Outcome Set-STAndards for Development
CROM	Clinician-reported outcome measure
DCC	Distal cervical caries
EQ-5D-3L	EuroQol five dimension three-level questionnaire
g (RCF)	Relative centrifugal force
GPBM	Generic preference-based measure
HIV	Human immunodeficiency virus
IAN	Inferior alveolar nerve
IOPA	Intraoral periapical
i-PRF	Injectable platelet-rich fibrin
ISTC	Independent sector treatment centre
L-PRF	Leucocyte and platelet-rich fibrin
L-PRP	Leucocyte and platelet-rich plasma
M3M	Mandibular third molar
MPQ	McGill pain questionnaire
MRONJ	Medication-related osteonecrosis of the jaws
NHS	National Health Service

NRS	Numerical rating scale
OHIP-14	14-item oral health impact profile
OHIP-49	49-item oral health impact profile
OHQoL-UK©	Oral health-related quality of life UK questionnaire
OMFS	Oral and maxillofacial surgery
OPG	Orthopantomogram
ORIF	Open reduction internal fixation
PCBM	Particulate cancellous bone marrow graft
POiS	Patient outcomes in surgery
PPD	Periodontal probing depth
PPP	Platelet-poor plasma
P-PRF	Pure platelet-rich fibrin
P-PRP	Pure platelet-rich plasma
PREM	Patient-reported experience measure
PRGF	Plasma rich in growth factors
PROM	Patient-reported outcome measure
PRP	Platelet-rich plasma
QoL	Quality of life
QOMS	Quality outcomes in maxillofacial surgery
RBC	Red blood cell
RCS Eng	Royal College of Surgeons in England
RCT	Randomised controlled trial
rpm	Revolutions per minute
SAC-MOT	Scientific advisory committee of the medical outcomes trust
SCTS	Society for cardiothoracic surgery
SF-12	12-item short form survey
SF-36	36-item short form survey
TMJ	Temporomandibular joint
TRMS	TheraBite® range of motion scale
VAS	Visual analogue scale
VDS	Verbal descriptor scale
VRS	Verbal response scale
WBC	White blood cell
WHO	World Health Organisation

ABSTRACT

Objectives

To investigate the effect of plasma rich in growth factors (PRGF) on clinician-reported and patient-reported outcomes following surgical removal of a unilateral impacted mandibular third molar.

Materials and Methods

Ethical approval to conduct this prospective, double-blind randomised controlled trial (RCT) was granted by the local Clinical Research Ethics Committee. Seventy-four patients requiring surgical removal of a single impacted mandibular third molar (M3M) under local anaesthesia were recruited to participate. A blood sample was obtained immediately pre-operatively (T0) for all participants irrespective of study arm allocation, and PRGF prepared according to the product protocol. Patients allocated to the treatment arm received PRGF clot in the third molar socket after tooth removal. All patients received a telephone call 3 days postoperatively (T1), and were asked to return to the clinic for review 7 days postoperatively (T2). Primary outcome measures were NRS (numeric rating scale) pain score, OHIP-14 (Oral Health Impact Profile-14) and PoSSe (Postoperative Symptom Severity) scale data. Secondary outcome measures such as mouth opening (MIO), dry socket, socket healing and analgesia consumption were also explored. Statistical analysis was performed using IBM SPSS® 25.0 software and Stata® 15.1. ANCOVA was used for analysis of NRS, OHIP-14 and PoSSe total

scores and MIO outcomes. Categorical variables were analysed using the Chi square test.

Results

The mean age of participants was 28.1years (range 19-39, SD 5.8) with females accounting for 77% of the study population. NRS scores were higher in the PRGF group at T1 (4.1±2.4) demonstrating borderline significance (p=0.06) with no significant difference at T2. No significant differences were observed in PoSSe subscales between groups overall, with the exception of the 'interference with daily activities' subscale at T1, with PRGF patients scoring on average 1.2units higher (p=0.02). OHIP-14 outcomes revealed patients in the PRGF group were 25% more likely to experience discomfort on eating at T1 (p=0.02) with no significant difference between groups at T2. Reduced MIO was observed at T2 in control (35.7±8.2) and PRGF groups (35.4±8.5), but was not significant (p=0.67). The incidence of dry socket was not significant between groups (p=0.3). Socket healing, graded using a modified Landry et al healing index, did not vary significantly between groups: control 4.0±1.2, PRGF 3.6±1.2, nor did analgesia consumption.

Conclusion

The results of this study did not demonstrate any significant difference in clinical or quality of life outcomes in patients following adjunctive use of PRGF in mandibular third molar sockets.

DECLARATION

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

_____

Laura O'Sullivan

ACKNOWLEDGEMENTS

CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

Third molar surgery accounts for the vast majority of oral surgery procedures performed worldwide (Hanna et al., 2018, Grossi et al., 2007, Jerjes et al., 2010, Ruta et al., 2000) and is one of the most popular research models for testing novel analgesics and various other interventions (Coulthard et al., 2014a). A recently published review article from our unit explored the lack of standardised methodology for outcome measurement in third molar surgery, despite an ever-expanding research output on the topic (O'Sullivan and Ní Ríordáin, 2021). It is hoped the publication will open a much-needed discussion on the development of core outcome sets not just for third molar surgery, but for the specialty of oral surgery as a whole.

Autologous platelet concentrate (APC) use was recognised for the first time in the 2020 third molar surgery Cochrane Review as a distinct surgical technique in its own right, although this review did not include plasma rich in growth factors (PRGF) in its discussion, instead focusing solely on PRP (platelet-rich plasma) and L-PRF (leucocyte and platelet-rich fibrin). Initial reports on the regenerative potential of APCs were published as recently as 1998, and despite their relative infancy, a huge body of evidence exists in support of their capacity to promote osseous and soft tissue regeneration through the physiological processes of platelet activation and subsequent growth factor release. In fact, APCs have transformed many areas of healthcare and are now considered an essential component of the surgical milieu.

Numerous studies to date have looked at APC use in mandibular third molar sockets, focusing primarily on postoperative pain experience, soft tissue healing and incidence of dry socket. To our knowledge, no study has yet investigated the impact on quality of life in this cohort using adjunctive APCs. With patient-reported outcomes playing an increasingly influential role in commissioning of healthcare services, quality of life evaluation is now widely accepted as an important tool in clinical research.

The literature review gives a comprehensive overview of third molars and third molar surgery as well as an in-depth look at the evolution of the various autologous platelet concentrates on the market today. Clinician-reported outcome measures are explored next, with a focus on recurring themes in the third molar literature. This is then followed by a discussion of patient-reported outcome measures, a relatively untapped resource in third molar surgery research.

1.1 CONFERENCE PRESENTATIONS, AWARDS AND PUBLICATIONS

Conference presentations (oral)

BAOS Annual Award Ceremony Open Paper Presentations, 2021 (Appendix A)
PRGF® use in third molar surgery: a randomised controlled trial

25th EACMFS Virtual Congress, 2021 (Appendix B)
Investigation of the effect of plasma rich in growth factors on quality of life following mandibular third molar removal: a randomised controlled clinical trial

Awards

1st prize winner Research paper category, BAOS Annual Award Ceremony Open Paper Presentations, 2021

Winner Open Paper People's Vote, BAOS Annual Award Ceremony Open Paper Presentations, 2021

Publications

O'Sullivan L, Ní Ríordáin R. Variations in clinician-reported outcome measures in third molar surgery: a focused review. *Surgeon.* 2021 May 10;S1479-666X(21)00074-3. DOI: 10.1016/j.surge.2021.03.008 (Appendix C)

O'Sullivan L, Ní Ríordáin R. Autologous platelet concentrates in oral surgery: protocols, properties and clinical applications. *OOOO – published online June 4th 2021.* DOI: 10.1016/j.oooo.2021.05.013 (Appendix D)

BTI site visit
Invited visitor to Biotechnology Institute, Vitoria-Gasteiz, Spain, 2019

CHAPTER TWO

LITERATURE REVIEW

2.1 THIRD MOLARS

2.1.1 Epidemiology

Third molar surgery accounts for the vast majority of oral surgery procedures performed worldwide (Hanna et al., 2018, Jerjes et al., 2009, Grossi et al., 2007, Ruta et al., 2000), with an estimated 152,000 patients treated on the National Health Service (NHS) each year in England alone (McArdle et al, 2018b). Although no equivalent data exist for the Republic of Ireland, third molar removal has long been established as the most commonly performed procedure by oral surgeons in this jurisdiction (Cowan, 2006). The majority of the adult population will admittedly be faced with undergoing this procedure at some stage throughout their adult lives (McGrath et al., 2003b).

2.1.2 Development and eruption

Radiographic signs of mandibular third molar (M3M) development can typically be expected around the age of 8 years (Gravely, 1965). The tooth 'bud' is initially located within the mandibular ramus, with the occlusal surface facing forwards and upwards at an average angulation of 38° to the mandibular occlusal plane (Richardson, 1970). What follows is a dual sequence of forward migration of the M3M bud and resorption of the anterior border of the ramus. As a result, the M3M then appears to be positioned within the body of the mandible (Fig 1). The bud then continues its journey of forward and upward migration towards the distal surface of the second molar and the alveolar crest, maintaining a mesial inclination

throughout. On reaching the distal surface of the adjacent second molar, the bud then undergoes a rotational movement and assumes a more upright position (Silling, 1973). Any deviation from this process of normal development will result in M3M impaction.

 Figure 1. Lateral oblique radiograph showing mesial orientation of the developing third molar in the body of the mandible (Silling, 1973)

2.1.3   Third molar impaction

An impacted tooth is defined as *"a tooth that is all the way or partially below the gum line and is not able to erupt properly"* (WHO, 2019) and is in essence, one that fails to assume a functional position within the dental arch. Impaction is recognised by the World Health Organisation (WHO) as a disease entity in its own right within the ICD-11 Classification of Diseases (DA07.8). Wisdom teeth (M3M) are the last tooth in the series to erupt into the oral cavity, typically between 18 and 24 years of age (McArdle et al., 2018a) and are the most commonly impacted of all teeth (Carr S, 2018). Winter's classification of third molar

impaction is commonly used by clinicians as a descriptive tool based on the radiographic inclination of M3M (Fig 2).
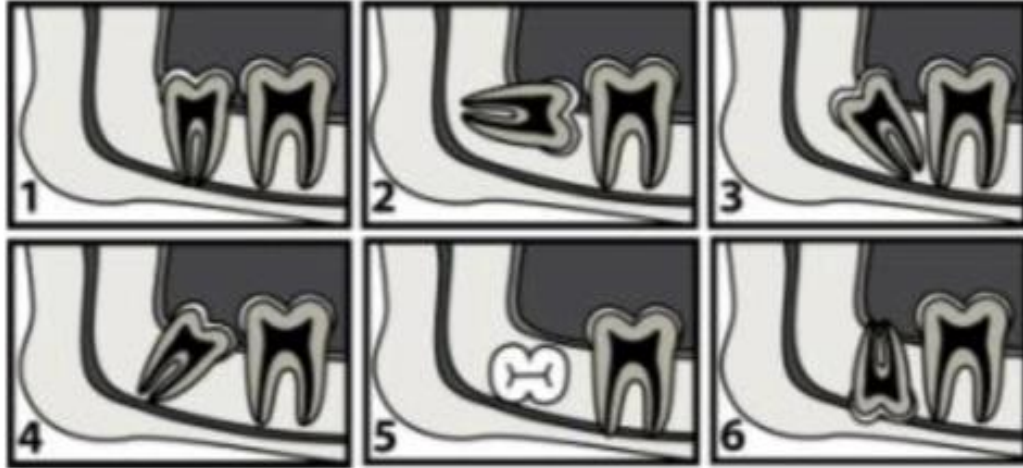
Figure 2. Winter's classification of mandibular third molar impaction. Third molars are classified according to their inclination relative to the long axis of the adjacent second molar. 1. Vertical, 2. Horizontal, 3. Distoangular, 4. Mesioangular, 5. Transverse, 6. Inverse (Miclotte et al., 2017)

The reported prevalence of third molar impaction in the 20-30 year age group is as high as 72.7% (McArdle et al., 2018a, Hugoson and Kugelberg, 1988). Previous operative removal renders observation of M3M impaction in older age groups less likely (FDS, 1997). A mesioangular orientation is observed most frequently in impacted mandibular third molars (McArdle et al., 2018a, Al-Anqudi et al., 2014), reflecting their developmental process.

The diseases most commonly associated with impacted M3M include:

- Pericoronitis

- Caries

- Periodontitis

- Distal cervical caries (DCC) of second molar

- Cyst formation

2.1.3.1 Pericoronitis

Pericoronitis is the single most common indication for lower third molar removal (McArdle et al., 2018a, van der Linden et al., 1995, Bruce et al., 1980). There are currently no internationally agreed diagnostic criteria for pericoronitis, but it is typically diagnosed where there is evidence of swelling of the soft tissues around an impacted third molar, and a history of food packing and purulent discharge from the associated soft tissues (Mackie et al., 2019). Although most cases of M3M-related pericoronitis will respond well to conservative management, there is a risk that more virulent infections will spread throughout the fascial spaces of the neck with potentially life-threatening consequences (Fig 3). Odontogenic infections are the most common cause of such a clinical presentation in adults (Main et al., 2016). Recent data from a tertiary referral centre in Australia reported a significant increase in the number of patients requiring urgent operative intervention and intensive care admission as a direct result of odontogenic infections, with M3M the most commonly implicated tooth in these cases (Fu et al., 2020).
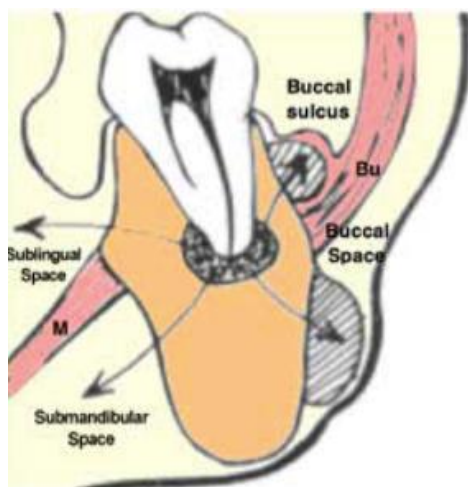
Figure 3. Potential routes of spread of infection from a mandibular molar tooth. M, mylohyoid muscle; Bu, buccinator muscle (Wali et al., 2020)

Recognition of the signs and symptoms of pericoronitis by the general dental practitioner, and onward referral where indicated, are imperative to ensure timely patient management and reduce cases of avoidable hospital admission.

2.1.3.2   Caries

Caries is the second most common indication for lower third molar removal, and is seen more commonly in older patients where the tooth has been in communication with the oral cavity for a period of time (McArdle et al., 2018a). Unfavourable tooth orientation and limited access are frequent barriers to restorative intervention in such instances.

2.1.3.3   Periodontitis

It is estimated that periodontitis accounts for 5% of all M3M extractions (McArdle et al., 2018a). In one retrospective analysis of 1,431 third molars removed in 1,011 patients, it was found that periodontal disease accounted for the removal of 13% of horizontally impacted M3M and 9% of vertical, non-impacted M3Ms (McArdle et al., 2018a).

2.1.3.4   Distal cervical caries (DCC) of second molar

Distal cervical caries (DCC) in the mandibular second molar is believed to be uniquely related to the mesioangular M3M and has been widely reported in the literature in recent years (McArdle et al., 2018a, McArdle and Renton, 2012, Allen

et al., 2009, Toedtling et al., 2019). DCC arises at the cementoenamel junction of the second molar and is considered a variant of root surface caries (Fig 4). No known cases of DCC have been reported in the absence of an adjacent mesioangular M3M (McArdle et al., 2014).

Figure 4. Radiograph of distal cervical caries in the lower right second molar with associated impacted mesioangular third molar (McArdle et al., 2014)

The degree of angulation of the mesioangular M3M is an important risk factor for DCC development, with an angulation of 40-80° to the mandibular plane most frequently observed (McArdle et al., 2014). In many cases, DCC is diagnosed at an advanced stage by which point the second molar is often deemed unrestorable. This contentious issue has been a topic of much research, and there is a strong argument in favour of prophylactic removal of mesioangular third molars to prevent those problems associated with their retention long-term (Allen et al., 2009, Toedtling et al., 2016).

2.1.3.5   Cyst formation

In some cases where an impacted M3M remains unerupted, cystic change may be observed. Dentigerous cysts are typically associated with unerupted teeth, and account for 20% of all odontogenic cysts (Hill and Renton, 2017, Daley et al., 1994). This cyst arises from separation of the reduced enamel epithelium once amelogenesis is complete and is believed to affect M3Ms almost ten times more frequently than their maxillary counterparts (RCSEng, 2020). Odontogenic cysts are often incidental findings with no presenting signs or symptoms; it is therefore prudent to monitor unerupted teeth on a regular basis to ensure any pathology is picked up at an early stage.

2.1.4   Guidelines

The Faculty of Dental Surgery at the Royal College of Surgeons of England convened a working party in 1997 for the development of guidelines on the management of third molar teeth (FDS, 1997). This document was reviewed in 2004, at which time it remained unchanged. A further decision to review the faculty's stance on third molar removal led to the publication of a revised document in 2020, with further edits in May 2021 (section 2.1.4.3).

In February 2015, The Scottish Intercollegiate Guidelines Network (SIGN) withdrew its document 'Management of unerupted and impacted third molar teeth' (SIGN, 2000) and for this reason will not be discussed further. The most robust guidelines currently available to clinicians are presented here.

2.1.4.1 National Institute for Health and Care Excellence (UK)

In March 2000, the National Institute for Health and Care Excellence UK (NICE) published its 'Guidance on the Extraction of Wisdom Teeth' document (TA1) in response to a finding that up to 44% of wisdom tooth extractions being undertaken were not justified on clinical grounds (NICE, 2000). The practice of prophylactic third molar removal has long since been discouraged, due to the morbidity associated with wisdom tooth removal, as well as the strain on health services that might otherwise be avoided (Fernandes et al., 2009).

NICE recommends removal of third molars only where a clear clinical indication exists. This includes two or more episodes of pericoronitis, unrestorable caries, non-treatable pulpal or periapical pathology, cellulitis, abscess and osteomyelitis, internal/external resorption of the tooth or adjacent teeth, fracture of tooth, disease of follicle including cyst/tumour, tooth/teeth impeding surgery or reconstructive jaw surgery, and where a tooth is involved in or within the field of tumour resection. A single severe episode of pericoronitis is also considered grounds for third molar surgery.

Since the introduction of these guidelines, there has been considerable debate as to their merit in situations where a malpositioned partially erupted M3M poses a real risk to the neighbouring tooth. Particular interest surrounds the enigmatic mesioangular impaction (Allen et al., 2009), which has been shown in numerous studies to lead to an increased incidence of DCC in the adjacent second molar tooth

(McArdle et al., 2018a, Toedtling et al., 2016, Toedtling et al., 2019, McArdle et al., 2014), often detected at a very late stage.

In one prospective cohort study looking at the course of 148 asymptomatic impacted M3Ms over a five-year period, authors concluded that 31% of these teeth required removal during the observation period (Hill and Walker, 2006). It has been shown that partially erupted teeth are twice as likely as unerupted teeth to develop symptoms following a 'watchful wait' period (Fernandes et al., 2009). Studies have shown a trend towards third molar surgery later in life, with an increase in the mean age of patients from 25 years in 2000 to 32 years in 2010 at the time of surgery (McArdle et al., 2018a). Increased age at the time of surgery is associated with increased surgical morbidity (Bruce et al., 1980).

In light of the inherent issues posed by the 2000 guidance that are reported in the literature, NICE responded in March 2015 by publishing an addendum to the TA1 document (NICE, 2015) urging clinicians to take into account the available guidance as well as *"exercising their judgment... alongside the individual needs, preferences and values of their patients"* and NICE guidance remains under review at the time of writing.

2.1.4.2   American Association of Oral and Maxillofacial Surgeons (AAOMS)

The White Paper produced by the American Association of Oral and Maxillofacial Surgeons (AAOMS) in 2016 (American Association of Oral and Maxillofacial

Surgeons, 2016) states that *"a decision should be made before the middle of the patient's third decade to remove or continue to observe third molars, with the knowledge that future treatment may be necessary based on the clinical situation."* In this document, AAOMS acknowledges the potential for future disease development related to disease-free third molars. It highlights the importance of the surgeon's role in determining the risk of future disease development in cases of asymptomatic impacted lower third molar teeth, and endorses the role of the surgeon in contributing to the decision-making process in the patient's best interests.

2.1.4.3   Faculty of Dental Surgery (UK) Guidelines

A guideline development group comprising a twelve-strong team of experts in their field, convened to review the pre-existing faculty guidelines on management of mandibular third molars (RCSEng, 2020). This review was undertaken *"because evidence suggests increasing patient harm due to retention of M3Ms."*

The consensus document 'Parameters of care for patients undergoing mandibular third molar surgery' was published in 2020 (and edited in May 2021), with the recommendation to shift *"from a solely therapeutic approach to a mixed range of interventions for patients with mandibular third molars based on a holistic and informed approach agreed with the patient"*. Particular emphasis is placed on patient education regarding the risks of retention, as well as surgical removal, of malpositioned M3Ms with special recognition of the evidence supporting prophylactic removal of the mesioangular M3M.

2.1.5 Morbidity

Patients undergoing third molar surgery will routinely be made aware of the common postoperative sequelae of pain, swelling and trismus (limitation of mouth opening less than 30mm) (Hill et al., 2001). Despite ample discussion regarding these sequelae, patients can often be surprised at the protracted morbidity associated with third molar surgery, with an associated deterioration in quality of life (QoL) reported for an average of five days post-operatively (McGrath et al., 2003b).

The clinical findings of swelling and trismus appear to be the main contributors to this deterioration in QoL. The effects of swelling and trismus on a patient's appearance and ability to masticate respectively, have been reported as the reasons for this finding (Savin and Ogden, 1997). These functional and aesthetic limitations have been shown to peak at day 1 post-operatively, with a gradual improvement towards baseline pre-operative levels by day 6 (Duarte-Rodrigues et al., 2018, McGrath et al., 2003b).

Pain is considered a reliable indicator of patient satisfaction (Coulthard, 2008), and may be influenced by factors such as patient anxiety, bone removal intra-operatively and operative duration (Coulthard, 2008, Hill et al., 2001). Studies have shown a peak in post-operative pain experience 3-5 hours following third molar surgery (Coulthard, 2008), which would certainly lend support to the aforementioned QoL deterioration during this period.

2.1.6   Complications of mandibular third molar surgery

Post-operative complications arise in up to 35% of cases of third molar surgery (Anjrini et al., 2014). The most common complication of any dental extraction, including third molar surgery, is alveolar osteitis (AO), more commonly referred to as dry socket. This is a localised bacterial infection, arising from anaerobic breakdown of the blood clot within the extraction socket, leading to persistent pain, malodour and delayed wound healing. Factors such as smoking, female gender, poor dental hygiene and concurrent oral contraceptive medication, increase the likelihood of developing dry socket post-operatively (Anjrini et al., 2014, Jerjes et al., 2010).

Those complications more specific to mandibular third molar surgery are discussed below.

2.1.6.1   Nerve injury

The posterior mandible is a high-risk site for dentoalveolar surgery, due in no small part to its complex anatomy. Specific to the lower third molar tooth is the close, and occasionally intimate, relationship between it and the lingual and inferior alveolar nerves. Iatrogenic trigeminal nerve injury is the most problematic complication of dentoalveolar surgery, with considerable medicolegal implications (Renton, 2010). Trigeminal nerve injuries can interfere with many day-to-day activities such as speaking, eating, smiling, kissing, shaving, make-up

application, toothbrushing and drinking (Renton, 2010). The negative impact on QoL, social interaction and general psychological wellbeing is well documented, with up to 70% of affected patients reporting long-term chronic pain and disability (Renton, 2017).

The incidence of lingual nerve injury has remained more or less unchanged over the last thirty years, ranging from 0.4-1.5% (Renton, 2013). Third molar surgery remains the most common cause (Renton, 2013). Factors that increase the risk of lingual nerve injury include the depth of impaction, lingual flap retraction, vertical tooth sectioning and operative duration (Valmaseda-Castellon et al., 2000, Hill et al., 2001). There is conflicting evidence to suggest that lingual nerve injuries are more likely to occur where there is an inexperienced operator and where surgery is performed under general anaesthesia (Brann et al., 1999); however, these speculations are not corroborated across the board. Recovery of lingual nerve injury within eight weeks is seen in 85-94% of cases (Renton, 2013) although spontaneous repair of sectioned lingual nerve axons is unlikely due to retraction and separation of nerve endings where there has been manipulation of the surrounding soft tissues (Loescher et al., 2003). Early referral to a specialist centre for investigation and prompt surgical intervention is therefore recommended, preferably within three months of the onset of injury. Access to the lingual nerve for surgical repair is considered favourable, due to its extraalveolar course as it runs deep to the lingual mucosa in the mandibular molar segments.   The lingual nerve lies above the level of the alveolar crest in 17.6% of patients (Hill et al., 2001, Kiesselbach and Chamberlain, 1984). The more proximal the site of lingual nerve injury, the lower the success rate for surgical intervention (Renton, 2013).

Third molar surgery accounts for up to 3.6% and 8% of permanent and temporary cases of inferior alveolar nerve (IAN) injury, respectively (Renton, 2012). Local anaesthetic administration, dental implant placement and endodontic treatment are also major contributors to IAN injury (Renton, 2013). The intraosseous anatomical course of the IAN within the confines of the mandibular canal, makes it less amenable to surgical repair compared to the lingual nerve (Renton, 2013). Recovery of sensation is unlikely beyond six months of the injury (Renton, 2013).

Trigeminal nerve injuries are more common in females, and show a positive correlation with operator inexperience. The highest incidence of IAN neurosensory deficit occurs in cases of horizontal M3M impaction (Hill et al., 2001), with the lowest incidence in vertical M3M impaction (Smith, 2013).

2.1.6.2   Mandible fracture

Iatrogenic fracture of the mandible is a rare, but potentially very serious, complication of third molar surgery, with a reported incidence of 0.0033 to 0.0049% (Libersa et al., 2002, Ethunandan et al., 2012). Males are twice as likely to be affected as females, with mesioangular M3Ms most commonly implicated (Ethunandan et al., 2012). Mandible fracture secondary to third molar removal is more likely to occur in patients who are in their fifth or sixth decade (Krimmel and Reinert, 2000). Management options include conservative management, offering soft diet advice and avoidance of contact sport for six weeks, closed reduction with orthodontic appliances or archbars, or open reduction and internal fixation with titanium plates and screws.

Comprehensive pre-operative assessment and appropriate radiographic investigations are essential for third molar surgical planning. The 'high-risk' radiographic signs of IAN injury risk are well-documented, and include narrowing of the IAN canal, deviation of the IAN canal around the M3M apices, a radiolucent band running across the M3M roots and an apparent disappearance of the IAN canal behind the M3M roots (Renton, 2012). Consideration should be given in such instances to ordering a cone-beam computed tomography (CBCT) scan to better evaluate the relationship of the IAN to the roots of the M3M of interest (Fig 5). This will facilitate clinical decision-making, and allow a more accurate discussion of nerve injury risk with the patient as part of the informed consent process. A deeply impacted M3M, close proximity to the lower border of mandible or association with cystic or other pathology will greatly increase the possibility of iatrogenic mandibular fracture and careful consideration should be given to alternative treatment options to mitigate these risks.
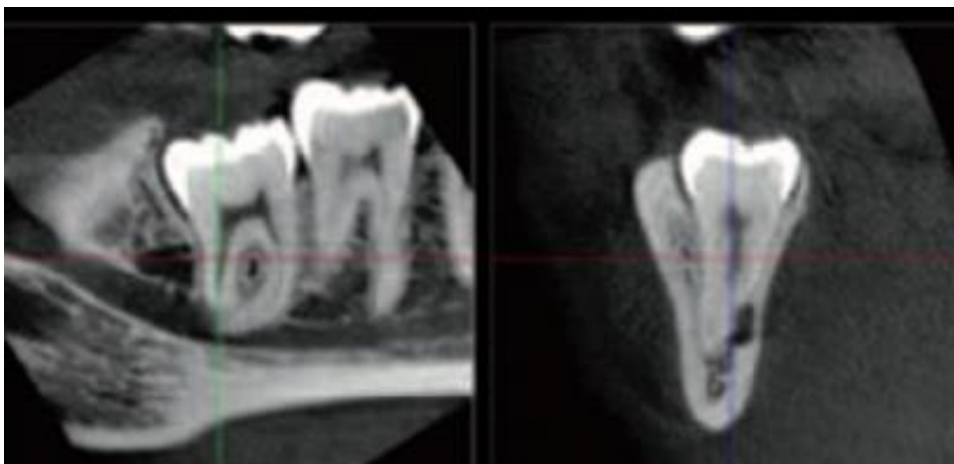
Figure 5. CBCT views showing concealment of the ID canal by the third molar roots (left) and decortication of the lingually positioned ID canal (right)

2.2 AUTOLOGOUS PLATELET-RICH THERAPIES

The last twenty years have seen huge advances in the development of autologous platelet-rich therapies, due in large part to their regenerative properties, as well as their wide availability, ease of use and cost effectiveness.

The use of autologous blood products in surgery was first reported in 1954 by Kingsley who used 'platelet-rich human plasma' for its haemostatic and adhesive properties (Kingsley, 1954, Mozzati et al., 2010). In 1970, Matras introduced the concept of fibrin glue by demonstrating enhanced healing of skin wounds in a rat model. She went on to describe the applications of fibrin glue in oral and maxillofacial surgery in 1982, notably enhanced tissue healing and haemostasis (Matras, 1982). Further reports by Matras in 1985 on the role of fibrin glue in microvascular and microneural surgery cemented the versatility and many benefits of this blood product. In 1994, Tayapongsak et al reported a 97% success rate in a cohort of thirty-three patients undergoing mandibular reconstruction with particulate cancellous bone and marrow (PCBM), using autologous fibrin adhesive (AFA) as a surgical adjunct to overcome the problem of separation of bone fragments during wound closure (Tayapongsak et al., 1994).

One of the many disadvantages of the fibrin glue system is the potential for blood-borne virus transmission, with at least one reported case of human immunodeficiency virus (HIV) transmission from a commercial fibrin glue system reported in the literature (Whitman et al., 1997, Wilson SM, 1991). Fibrin glue relies on the concentration of a large quantity of fibrinogen, which in the presence

of calcium is cleaved by thrombin in the final coagulation cascade to form fibrin (Fig 6). Factor XIII, once activated by thrombin, crosslinks fibrin strands to form an organised clot. This technique requires the use of exogenous bovine thrombin to activate the process (Mozzati et al., 2010, Tayapongsak et al., 1994). Fibrinogen is sourced from either donor cryoprecipitate, or autologous plasma, which requires patient attendance at the blood bank at least three days, and in some cases up to three weeks, prior to surgery. Today, autologous fibrin glue production remains limited due to the associated costs and complexity of production. Instead, commercial products made from (non-autologous) human plasma such as Tisseel (Baxter, USA) are widely used (Dohan Ehrenfest et al., 2009).
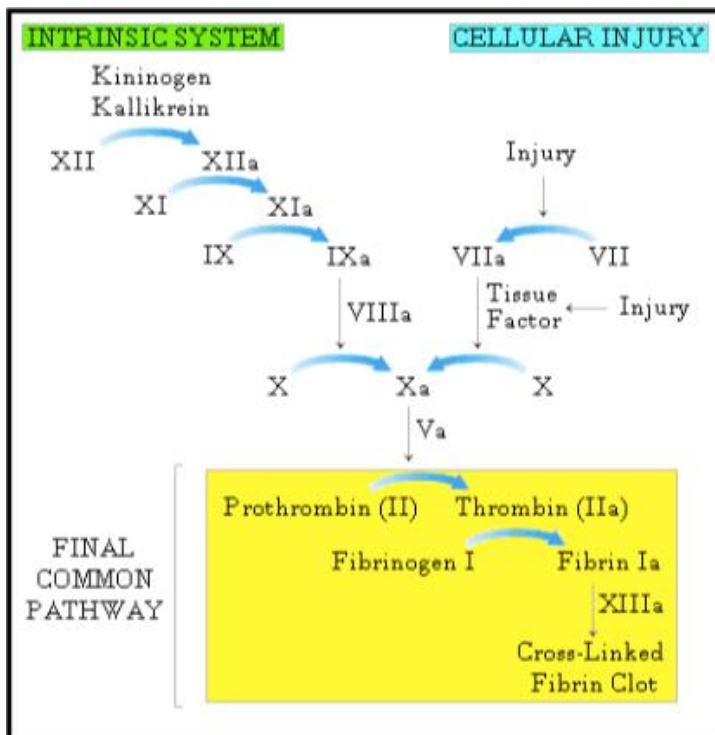
Figure 6. Coagulation cascade (Prosciak and Stawicki, 2017)

In an effort to address the shortcomings of fibrin glue, Whitman et al in 1997 described platelet gel as a more favourable autologous alternative. The critical difference between the two is that the latter has a high concentration of platelets

and a native concentration of fibrinogen. Whereas fibrin glue preparation requires pre-donation of blood up to three weeks prior to surgery, the platelet gel technique involves collection of one unit of whole blood (450mL) immediately preoperatively. The standard blood collection bag is labelled, and contains citrate-phosphate-dextrose anticoagulant. Whitman et al document a two-stage centrifugation process in the preparation of platelet gel. The first cycle spins the blood at 5,600rpm to separate the platelet-poor plasma from the erythrocytes and 'buffy coat' in which leucocytes and platelets are suspended. The platelet-rich plasma is obtained by a slower second centrifugation cycle at 2,400rpm, which further separates the buffy coat from the haematocrit (Fig 7).
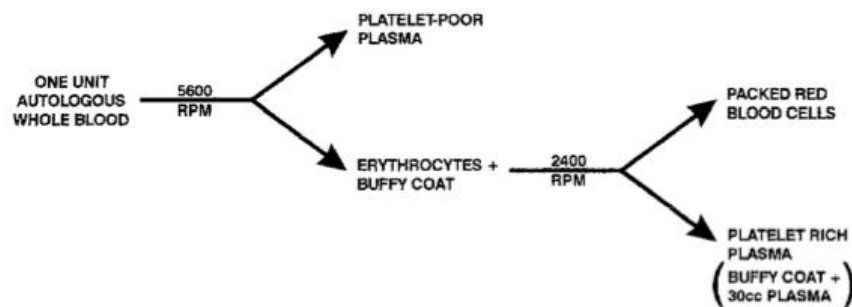
Figure 7. Preparation of platelet gel (Whitman et al., 1997)

The next step is to collect 7mL of the platelet-rich plasma into a syringe, together with 2mL of air. Bovine thrombin (10,000 units) in powder form is dissolved in 10mL 10% calcium chloride in a sterile cup, and 1mL of this mixture is aspirated into the syringe. Gentle rocking of the syringe allows the air bubble to disperse the contents evenly along its length, ensuring an even mixture. The resulting gel is injected directly from the syringe onto the surgical site as required. The authors do not disclose the centrifugation times required for the protocol. The reported

thirty minutes total preparation time would appear ambitious for even the most efficient of operators.

This initial inception of autologous blood products into the surgical armamentarium sparked much further research into the selective sequestration of platelets from autologous blood samples, and the possibility that these resulting platelet concentrates might confer a physiological advantage in the healing of hard and soft tissue defects. And so was born the era of autologous platelet-rich therapies.

2.2.1 Platelet-rich plasma

Following on from the earlier work of Whitman et al into the standardisation of autologous platelet gel preparation, Marx et al took things a step in further in 1998 by investigating and reporting more specifically on the role of platelet growth factors in the healing and regenerative mechanisms with respect to mandibular bony reconstruction. They employed the same two-step centrifugation process described earlier by Whitman et al, but outline the cell separation process in more detail. The centrifugation process generates three distinct layers: around 180mL red blood cells/haematocrit, 70mL platelet-rich plasma ("buffy coat") and 200mL platelet-poor plasma (Fig 8). The platelet-poor plasma component is removed manually using a pipette, and a slower second centrifugation cycle is then undertaken to allow further separation of the buffy coat from the haematocrit. In their cohort study of eighty-eight patients, each requiring reconstruction of mandibular defects ≥5cm using donor posterior ilium PCBM, they reported

accelerated and enhanced bone deposition in those patients who had adjunctive treatment with PRP at the graft site. Histomorphometry conducted six months post-operatively showed greater trabecular bone density in patients whose grafts were treated with PRP (Marx et al., 1998).
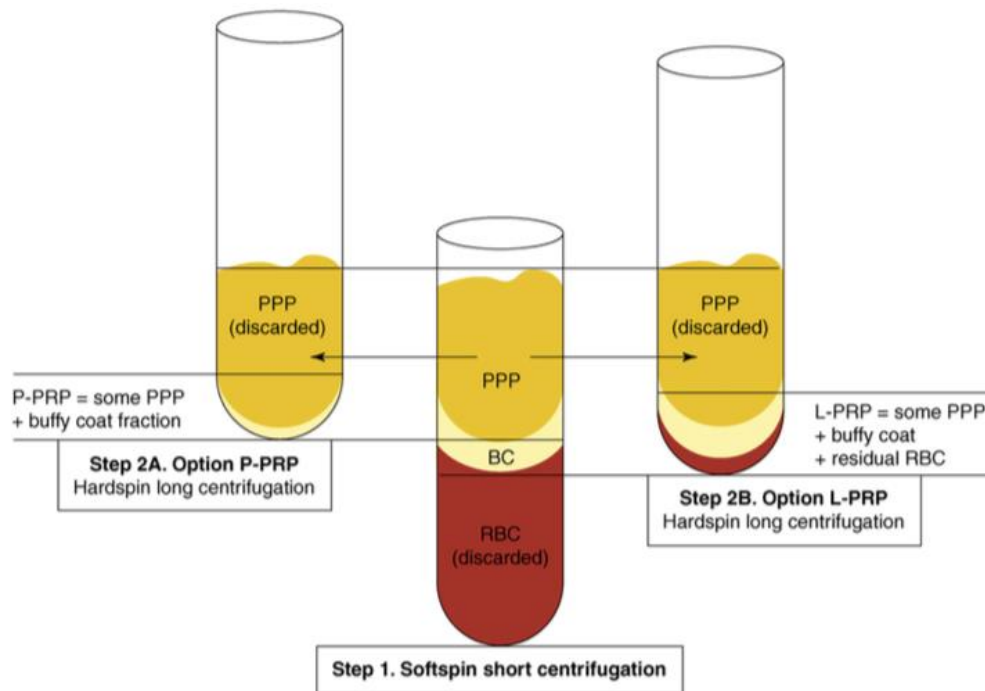
Figure 8. Manual platelet-rich plasma (PRP) protocol using a two-step centrifugation process (Dohan Ehrenfest et al., 2009)

The enhanced bone deposition observed in the study was attributed to the synergistic interplay of growth factors secreted by platelet α-granules and the corresponding receptors on the membranes of marrow stem cells and osteoprogenitor cells present in the cancellous marrow grafts (Marx et al., 1998). Identification of this native concentration of autologous growth factors within platelet concentrates was an undisputed breakthrough in the area of regenerative medicine.

PRP deserves recognition for paving the way for further advances in autologous platelet therapies; indeed, Marx et al succeeded in standardising the preparation protocol in line with that previously described by Whitman et al. The inconvenience of patients having to attend several days or weeks prior to surgery to donate a blood sample was overcome by obtaining blood samples immediately pre-operatively. Both platelet gel and PRP permit a 'command gelification' by the manual addition of a calcium-based activator to the platelet concentrate. This allows surgeons to apply PRP/platelet gel as required to the surgical site at the desired time.

While the above advances cannot be disputed, the following factors negatively impact the accessibility, affordability, acceptability and adaptability of the technique for use in primary care:

- Large quantity of blood (400-450mL) required to produce approximately 70mL platelet-rich plasma

- Complex cell separator system such as Electro Medics 500 (Medtronics) (Marx et al., 1998), often requiring help from a haematologist

- Additional costs of catheters, central venous lines, internal centrifuge bowl amounting to hundreds of dollars

- Two centrifugation cycles, making the process time-consuming

- Use of bovine thrombin together with calcium chloride to activate the final common pathway of the coagulation cascade, corresponding to the gelification phase of the PRP protocol

The final addition to the first-generation family of platelet concentrates is a preparation known as plasma rich in growth factors (PRGF). This was the first system to effectively open the door to the use of autologous platelet therapies in a dental practice setting, and is the focus of this research project.

2.2.2 Plasma rich in growth factors (PRGF)

Anitua and his team at Biotechnology Institute (BTI) in Vitoria-Gasteiz, northern Spain, have been conducting ongoing research over the past twenty-five years in the fields of regenerative medicine and dental implantology (Fig 9). The therapeutic benefits of plasma rich in growth factors (PRGF) were first reported by Anitua in 1999, specifically in relation to accelerated bone regeneration and soft tissue healing around dental implant fixtures following adjunctive treatment with PRGF (Anitua, 1999). This product is developed commercially by BTI under the trademark Endoret®, and is sold worldwide with offices in mainland Europe, United Kingdom, North America and Mexico (BTI).

Figure 9. Eduardo Anitua Institute, BTI, Vitoria-Gasteiz, Spain
(Photograph taken by author 11th June 2019)

The BTI headquarters in Vitoria-Gasteiz comprise surgical facilities, research laboratories and classrooms for hosting domestic and international delegates with a special interest in BTI products such as Endoret®, UnicCa® implants and Scan®3 software for implantology planning. It has in fact become customary for existing and prospective clients, or those with a research interest in BTI technology, to be hosted at the Eduardo Anitua Institute for comprehensive didactic teaching with Dr Anitua and his team of experts. These are typically two-day visits, with interactive small-group lectures, hands-on preparation of Endoret®, placement of UnicCa® implants in bovine bone, and first-hand observation of Dr Anitua operating on live patients (Figure 10a-d).

Figure 10 (a-d). (clockwise from top left) **a.** Hands-on preparation of Endoret® prgf **b.** Placement of UnicCa® implant in bovine bone **c.** Social gathering at Le Bost on final night of visit (Dr Eduardo Anitua pictured front left) **d.** Classroom teaching using Scan®3 planning software

Profits from commercial sales go back into the training and research centres at BTI to help finance ongoing training of medical and dental personnel, and support the

prolific research output generated by the institute. BTI is involved in ongoing collaborations with several prestigious universities including Turin, and Harvard and Tufts in Massachusetts, USA.

2.2.2.1 Endoret® preparation and technique

A clear and descriptive Endoret® preparation protocol (Appendix E) is available to view on the BTI website, and has been described in numerous publications (Mozzati et al., 2010, Dohan Ehrenfest et al., 2009, Anitua et al., 2015b, Anitua et al., 2015a, Nishiyama et al., 2016, Khorshidi et al., 2016, Haraji et al., 2012). The basic equipment required for the production of Endoret® PRGF includes: system V centrifuge, plasmaterm H, work rack, activation containers and digital timer unit, all of which are reusable. One single-use KMU15 kit is required per patient, and contains four blood collection tubes, two fractionation tubes, one ampoule of activator, plasma transfer device, butterfly venepuncture apparatus and identification labels (Fig 11). The system as a whole is relatively inexpensive (Dohan Ehrenfest et al., 2009).

Figure 11. Endoret® preparation equipment, from left to right: centrifuge V, plasmaterm H, work rack, activation dishes
(accessed at en_endoret_catalogo_aparato_locomotor.pdf)

Endoret® preparation can be broadly categorised into four stages, listed below and

summarised in Table 1:

- Blood collection

- Centrifugation

- Fractionation

- Activation

STEP 1: Blood collection	STEP 2: Centrifugation	STEP 3: Fractionation	STEP 4: Activation
• A total of 36mL of venous blood is collected in 9mL tubes • Blue-capped tubes contain 3.8% sodium citrate as anticoagulant • Grey-capped tubes without anticoagulant are also available	• Tubes are inserted into the PRGF system V centrifuge, correctly balanced • Blood is centrifuged at 580g for 8mins at room temperature • Centrifuged blood samples are then carefully transferred to a work rack	• Lines are drawn on each tube as follows: ○ 0.2-0.3mL above the RBCs to mark the limit of the leucocyte layer ○ 2mL above this line to delineate the platelet-rich layer (fraction 2) from the platelet-poor plasma (fraction 1) • A plasma transfer device (PTD) is used to collect fraction 1 and fraction 2 components into labelled individual white-capped tubes	• Each mL of plasma is activated by addition of 2units of calcium chloride • Fraction 1 and fraction 2 suspensions are poured into individual glass dishes and placed in the Plasmaterm H device for around 10mins • Activated fraction 1 forms a fibrin membrane • Activated fraction 2 forms a jelly-like 'clot'

Table 1. Summary of Endoret® preparation steps using KMU15 kit (BTI)

2.2.2.1.1 Blood collection

Venous blood is collected into four 9mL tubes, each containing 0.2mL sodium citrate anticoagulant. This is carried out using the butterfly needle included in the KMU15 kit. The four tubes are placed in the system V centrifuge in a balanced manner to evenly distribute the weight; in the event an odd number of tubes is obtained, an empty tube should be filled with water to correct the imbalance. Ideally, centrifugation should take place shortly after, and no more than sixty minutes after, blood collection.

2.2.2.1.2 Centrifugation

The System V Centrifuge is automated to centrifuge blood samples at 580g for 8 minutes. Unlike the original PRP protocol, Endoret® is a single spin system. The slower centrifugation speed used for PRGF preparation is believed to result in fractionation of the various blood cell constituents mainly by their specific gravities (Nishiyama et al., 2016); these values are summarised in Table 2. Based on this principle, the theoretical order of the individual fractions from the bottom of the tube to the top is the red cell fraction or haematocrit, white cell fraction and platelet fraction, respectively (Figure 13). Overlapping of the individual fractions at slower centrifugation speeds is rarely seen; conversely, higher centrifugation speeds such as those seen in PRP preparation can promote the interplay of external influences such as cell size, deformability and fluid viscosity resulting in suboptimal separation of the various cell fractions.

Blood constituent	Specific gravity
Red blood cells	1.095-1.101
White blood cells	1.055-1.095
Platelets	1.058
Plasma	1.024-1.030

Table 2. Specific gravities of the various blood constituents (Nishiyama et al., 2016)

Nishiyama et al liken shear flow within blood vessels to higher centrifugation speeds, both of which can cause red cells to migrate away from the vessel or tube walls due to their deformability, while platelets collect along the walls of the vessel or tube (Figure 12).

The simplified, single-step centrifugation process described by Anitua et al results in better defined layers or fractions with less crossover, optimising the separation of the buffy coat from the platelet-rich 'fraction 2' component immediately above. This near-total elimination of leucocytes from the product is the hallmark of Endoret®.
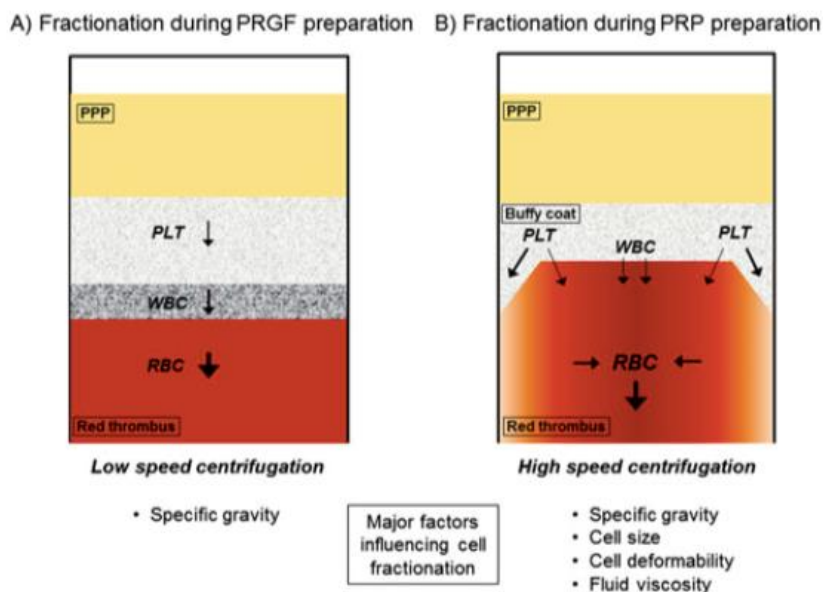
Figure 12. Representation of wall-normal force and volume exclusion that may occur in a blood tube during fast centrifugation of PRGF and PRP (Nishiyama et al., 2016)

2.2.2.1.3 Fractionation

Some authors have criticised the Endoret® fractionation method, dismissing it as mere 'eyeballing'. These same authors report inconsistencies in the volume of PRGF produced between patients, citing reproducibility challenges as a major disadvantage of the Endoret® system (Dohan Ehrenfest et al., 2009). These reports are at odds with findings of a consistent 2mL volume of platelet-rich 'fraction 2' immediately above the buffy coat following centrifugation (Anitua et al., 2015b, Anitua et al., 2015a), (Fig 13). The haematocrit volume, which is highly variable between individuals, is inversely related to the volume of platelet-poor 'fraction 1' and vice versa. Fraction 2 volume, however, is not influenced by the haematocrit (Anitua et al., 2015a). The clear steps outlined in the Endoret® protocol would also appear to contradict the reproducibility issues reported by Dohan Ehrenfest et al (Appendix E).

On retrieval of the tubes from the centrifuge, the tops are removed from each of the four tubes, and markings placed with an ink pen as shown in Figure 13 to demarcate the haematocrit (erythrocytes), buffy coat (leucocytes), fraction 2 (platelet-rich component) and fraction 1 (plasma-poor component). Fractionation is carried out using a plasma transfer device (PTD) to collect all fraction 1 from each of the four tubes into a separate white-capped tube in the first instance. Once this is complete, fraction 2 from each tube is then collected using the PTD into a separate labelled white-capped tube. The fractionation process should be carried out immediately after centrifugation to prevent diffusion-based loss of separation of the various fractions.
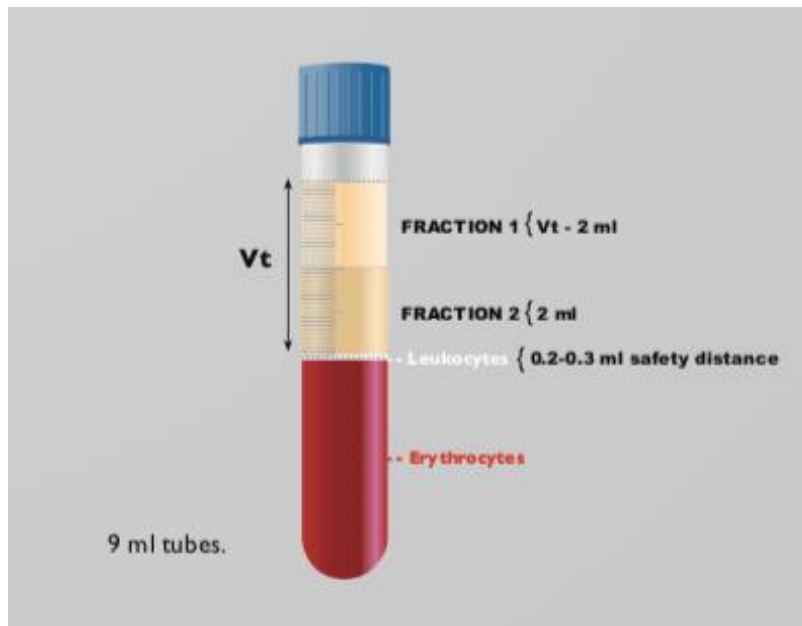
Figure 13. Centrifuged blood sample with various fractions labelled

2.2.2.1.4 Activation

The final step in the preparation of Endoret® is the addition of 10% calcium chloride activator to fraction 1 and fraction 2. The amount of activator is dependent on the volume of plasma in each fraction, with one unit of activator added per 0.5mL plasma; assuming a total volume of 8mL fraction 2 is collected during preparation, 16units of calcium chloride are added to the tube to activate it.

Activation with calcium chloride brings about the formation of native thrombin, which forms a three-dimensional fibrin 'clot', as well as platelet aggregration and degranulation, releasing numerous growth factors that become enmeshed in the fibrin scaffold (Fig 14).

Figure 14. Photograph showing platelet-rich fibrin 'clot' (fraction 2) (Anitua et al., 2006) (bar =5mm)

Once the activator is added, each fraction is poured into a separate 'activation dish' (Fig 11) and heated in the plasmaterm H device at body temperature for 10-15minutes. The fraction 2 'clot' is rich in platelets and assumes a jelly-like consistency, while the fraction 1 'membrane' is a condensed fibrin structure with far less cellular entrapment. In a study conducted by Anitua et al to investigate the effects of anticoagulant and antiplatelet drugs on the preparation of PRGF, they found that for patients taking warfarin, time to clot formation was significantly longer compared to the control group. For the five warfarinised patients in their study, mean time to clot formation was 43.3mins, compared to 19.7mins for the control group (Anitua et al., 2015b). It has been suggested that a warfarin-induced reduction in coagulation factor synthesis may have a knock-on effect on thrombin production (Fig 6), thus prolonging the activation phase of the PRGF preparation process.

2.2.2.2 Properties

Selective exclusion of leucocytes from the platelet concentrate is the hallmark of Endoret® preparation. Several schools of thought exist as to whether leucocytes should or should not be included in autologous platelet products, with no agreed consensus. One argument for exclusion of leucocytes is the production of a more homogenous and reproducible platelet product (Anitua et al., 2006). An in vitro study investigating the activity of human fibroblasts and osteoblasts treated with PRP and PRGF, under both normal and inflammatory conditions, showed consistently elevated release of pro-inflammatory cytokines such as IL-6, IL-8, TNF-α and IL-1β in the PRP-treated cell groups. Although cytokines have a role to play in the inflammatory process and in fighting infection, excessive production can be destructive to surrounding tissues (Anitua et al., 2015c). Concerns have also been raised about the potential for extracellular matrix destruction by neutrophils due to the release of matrix-degrading enzymes such as matrix-metalloproteinase-8 (MMP-8) and MMP-9, as well as reactive oxygen species that destroy healthy as well as injured tissues (Anitua et al., 2006).

Nishiyama et al investigated the composition of PRGF fraction 2 versus PRP by collecting venous blood from seven healthy non-smoking volunteers. Using an automated haematology analyser, they were able to show near total elimination of RBCs and WBCs from PRGF preparations, whereas the WBC count was increased 5.5-fold in the case of PRP (Fig 15). Platelets were concentrated by a factor of 2.84 in PRGF, and 8.79 in PRP, while actual numbers of platelets per preparation were slightly higher in the former.

PRGF exerts its regenerative effects at sites of injury or surgery by the release of growth factors from activated platelets. These growth factors in turn attract a native concentration of osteoblasts and fibroblasts, helped by the formation of a biological three-dimensional fibrin scaffold during the activation phase, which helps to localise these cellular components and growth factors at the site. Optimisation of cellular proliferation has been the focus of much research, with one in vitro study demonstrating optimum proliferation of fibroblasts and osteoblasts at a platelet concentration 2.5 times that seen in whole blood. Concentrations of platelets above this level showed a negative effect on cellular proliferation and impairment of osteoblast function (Graziani et al., 2006). Nishiyama et al's findings confirm platelet concentrations in PRGF are in the optimum range for maximum regenerative potential.
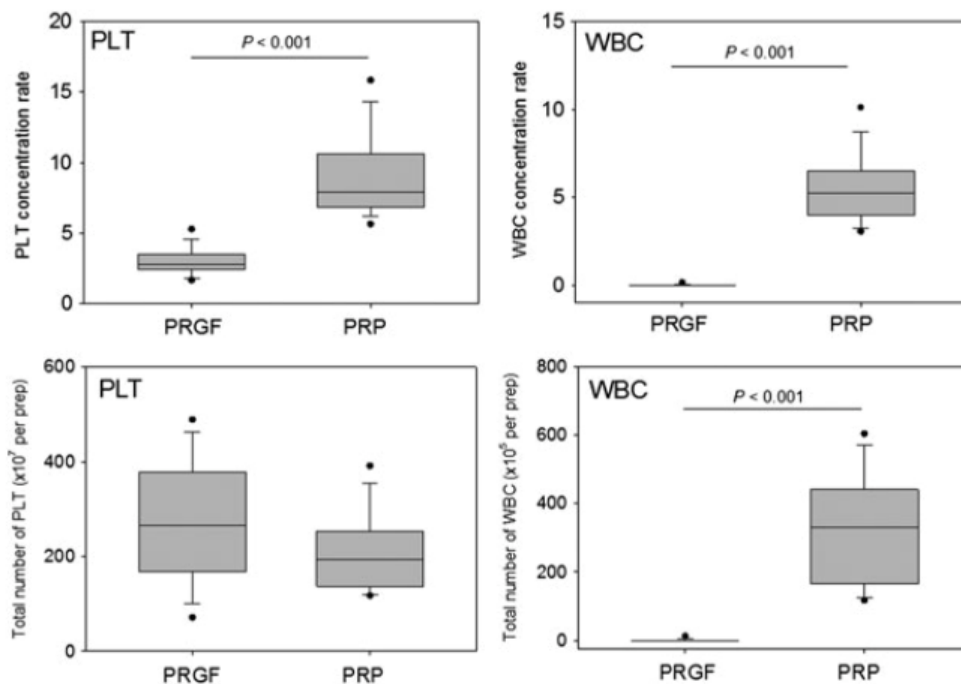
Figure 15. Box plot representation of concentration rates and cell compositions of PRGF and PRP fractions. WBC, white blood cell; PLT, platelet; PRGF, plasma rich in growth factors; PRP, platelet rich plasma (Nishiyama et al., 2016)

The potential antibacterial effects of PRGF against methicillin-sensitive and methicillin-resistant strains of *Stapylococcus aureus* (MSSA, MRSA) and *Staphylococcus epidermidis* (MSSE, MRSE) have been studied in vitro (Anitua et al., 2012). Although wound infections are typically polymicrobial in nature, staphylococci are believed to be major players in the aetiology of delayed healing and infection in both acute and chronic wounds (Bowler et al., 2001). Using blood samples from five healthy volunteers, Anitua et al prepared three different formulations of PRGF: fraction 1 (F1, platelet-poor, leucocyte-free), fraction 2 (F2, platelet-rich, leucocyte-free) and fraction 3 (F3) containing leucocytes from the buffy coat. A strong bacteriostatic effect against MRSA, MSSA and MRSE was demonstrated by all PRGF formulations for the first four hours. MSSE was less susceptible to the antimicrobial activity. After eight hours, the staphylococcal strains tended to recover once again. The leucocyte-rich F3 formulation showed superior bacteriostatic properties against MRSA compared to F2, but no difference compared to F1. Similar results have been reported for in vitro studies of PRP, which showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Bielecki et al., 2007).

This antibacterial property has been attributed to the release of cationic antimicrobial peptides from platelets, known as platelet microbicidal proteins (PMP) at sites of tissue injury or infection. Although the antibacterial effect of PRGF has yet to be fully evaluated in vivo, it is reasonable to extrapolate the role that PRGF preparations might play in prophylaxis against surgical site infection.

Whether this in vivo evidence can be applied in the prophylaxis against infection specifically in the oral cavity is yet to be fully elucidated, with only weak evidence currently available to support the role of APCs in the prevention of dry socket (Bailey et al., 2020). The oral cavity is home to over 700 different species of oral commensals, and it is long established that *Streptococcus viridans* is the major player in the aetiology of odontogenic infections (Donkor and Kotey, 2020). While there are reports in the literature of *Staphylococcus aureus* carriage rates of between 24-84% in the oral cavities of healthy, dentate individuals, it is generally accepted that preferential colonisation of the anterior nares is the norm (Donkor and Kotey, 2020). Furthermore, MRSA appears to preferentially colonise denture surfaces over healthy oral mucosa. Further evidence is required to fully explore the antibacterial properties of APCs in the context of prophylaxis against surgical site infection within the oral cavity.

2.2.2.3 Platelets and growth factors

Platelets are small anucleate cell fragments derived from megakaryocytes (Yadav and Storrie, 2017) with a lifespan of 9-10 days (Ogundipe et al., 2011). They are the smallest of the blood cells with an average diameter of 2-5μm and a thickness of 0.5μm, and number $150\text{-}400 \times 10^9$ in the average individual (Gremmel et al., 2016). The role of platelets in haemostasis and thrombosis was recognised as far back as 1882 by Bizzozero, who described the adherence of platelets to sites of blood vessel injury and formation of platelet aggregates to begin the repair process (de Gaetano and Cerletti, 2002).

The internal structure of platelets has been studied at length with the aid of electron microscopy, cell fractionation and platelet release studies (Yadav and Storrie, 2017). Platelets contain many of the cytoplasmic organelles common to most eukaryotic cells including mitochondria, endoplasmic reticulum (termed dense tubular system DTS in platelets), autophagososomes, endosomes and lysosomes (Fig 16). In contrast to most other cells, these organelles are primarily secretory in function, releasing their contents readily in response to signals such as collagen, thrombin and thromboxane A2. In the 1960s, two additional platelet-specific secretory organelles were identified as being central to the processes of haemostasis, thrombosis, inflammation, angiogenesis, host defence and mitogenesis:

- α-granules
- dense (δ) granules

Granule formation occurs in the megakaryocyte precursor, with maturation continuing in the circulating platelet (Gremmel et al., 2016).
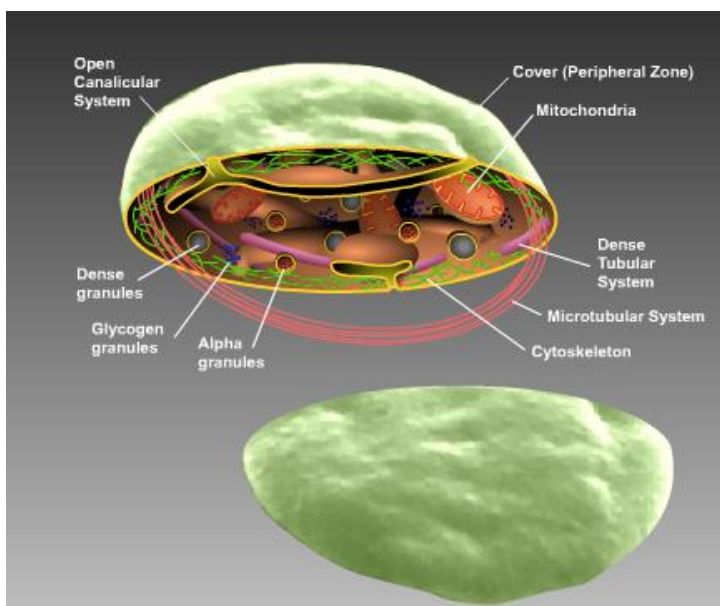
Figure 16. Internal platelet structure http://www.platelet-research.org/

2.2.2.3.1 α-Granules

α-granules are the most abundant of all platelet organelles, with the average platelet boasting between 50-80. These granules have a round to oval shape and a diameter of 200-500nm. They play a fundamental role in protein synthesis, storage and release. Native proteins are synthesised by the endoplasmic reticulum, and transported to the Golgi complex where they are packaged in immature granules (Gremmel et al., 2016). External plasma proteins such as fibrinogen, on the other hand, are taken up by endocytosis. All of these proteins are either suspended within the α-granule or bound to its membrane. One example of the latter is P-selectin which is exclusively expressed in activated platelets, and therefore used as a common cytometric marker of platelet activation.

Type	Examples
Integral membrane proteins	P-selectin GPVI
Coagulants, anticoagulants, fibrinolytic proteins	Factors V, IX, XIII Antithrombin Protein S Tissue factor pathway inhibitor Plasminogen
Adhesion proteins	Fibrinogen Von Willebrand factor Thrombospondin
Growth factors	Epidermal growth factor (EGF) Hepatocyte growth factor (HGF) Insulin-like growth factor (IGF) Transforming growth factor β (TGF-β)
Angiogenic factors and inhibitors	Vascular endothelial growth factor (VEGF) Fibroblast growth factor (FGF) Platelet-derived growth factor (PDGF) Angiostatin Endostatin
Chemokines	CXCL1, CXCL4, CXCL5, CXCL8, CCL2, CCL3, CCL5
Microbicidal proteins	Thymosin- β4 Thrombocidins 1 and 2
Immune mediators	Complement C3, C4 precursors IgG

Table 3. α-granule contents (Gremmel et al., 2016)

2.2.2.3.2 Dense granules

Dense granules are less populous than their α-granule counterpart, with an average of 3-8 per human platelet. They are also smaller in size, and tend to exhibit greater morphological heterogeneity. Dense granules are produced by the platelet endosomal system, and are so-named due to the increasing density of their content as they mature. They are the primary source of adenosine diphosphate (ADP) during haemostasis, one of the main drivers of platelet aggregation and activation (Gremmel et al., 2016).

Their contents include:

- nucleotides: ADP, adenosine triphosphate (ATP), uridine triphosphate (UTP), guanosine triphosphate (GTP)
- bioactive amines: serotonin, histamine
- phosphates: polyphosphate, pyrophosphate
- cations: Ca^{2+}, Mg^{2+}, K^+

2.2.2.3.3 Platelet Activation

Platelets are activated by a number of different mechanisms, the classical scenario being a contact-induced interplay between exposed collagen in the wall of a damaged blood vessel and platelet surface receptors, with simultaneous binding of vWF to the platelet surface glycoprotein complex.

This process stimulates migration of α- and δ-granules to release their contents either directly via fusion with the platelet membrane, or indirectly via exocytosis into the open canalicular system and subsequent release into the extracellular space. The flood of local coagulation factors, particularly factor V, promotes production of thrombin via the final common pathway of the coagulation cascade (Fig 6). A concomitant increase in intracellular Ca^{2+} concentration promotes further granule secretion.

Giving special consideration to the activation process of PRGF, addition of 10% calcium chloride triggers an increase in local thrombin formation. Thrombin is believed to be the most potent platelet activator, exerting its effect by binding to GP1b-IX-V and a group of protease-activated receptors (PARs). Of the four PARs, only PAR-1 and PAR-4 play a role in platelet activation, with the former being the most important player in the process. PAR-4 responds only to very high levels of thrombin, whereas PAR-1 is sensitive even to very low thrombin levels (Gremmel et al., 2016).

The surface area of a platelet can increase by a factor of four during this activation phase, by development of numerous cellular extensions or pseudopodia, as well as evagination of the open canalicular system channels. The resulting increase in receptor availability complements the entire process. During this morphological transformation, platelet granules accumulate in the cell centre in a process known as 'centralisation' and in some instances coalesce with other granules (Fig 17). The simultaneous release of growth factors, coagulation factors and fibrinogen from α-granules prompts a sequence of angiogenesis and cellular events, thrombin

production and formation of a stable fibrin clot, respectively. Over 300 different proteins have been identified during α-granule secretion (Gremmel et al., 2016). At the same time, dense granules release a host of proteins each with important effects: ADP enhances platelet-platelet aggregation, serotonin enhances vascular tone, and Ca^{2+} ions promote thrombin formation and further granule secretion.
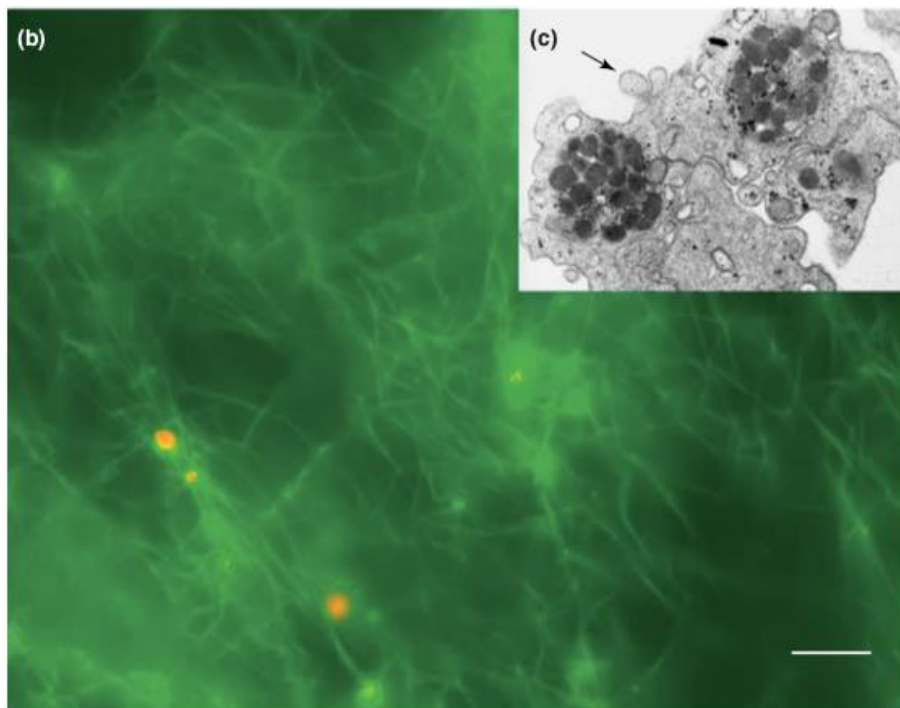
Figure 17b. Structure of the platelet-rich fibrin clot as seen on fluorescence microscopy showing a network of fibrin strands (green fluorescence) and platelet aggregates (yellow-red fluorescence) (bar = 40μm) **c.** Transmission electron micrograph of a platelet aggregate showing signs of activation, including the centralisation of granules and pseudopod extrusion (arrow) (Anitua et al., 2006)

This complex interplay of platelet activation, fibrinogen release, thrombin production and growth factor secretion culminates in the formation of a stable fibrin clot. Endothelial cell proliferation occurs in response to the release of VEGF, essential for the process of angiogenesis, while fibroblasts and osteoprogenitor cells migrate to the site in response to TGF-β, PDGF and FGF secretion from α-granules.

2.2.2.3.4 Growth factors

Growth factors are fundamental to the successes of regenerative medicine, being hailed as a 'biological solution to biological and medical problems' (Anitua et al., 2007b). The rationale behind the development of PRGF was that by eliminating RBCs and WBCs, the resulting platelet-enriched preparation would not only enhance, but also accelerate, the overall healing process (Anitua et al., 2007b). During the activation phase of PRGF preparation, fibrinogen released from α-granules is converted to fibrin fibrillae by thrombin. These fibrillae assemble in a tetra-molecular three-dimensional structure (Giannini et al., 2015). In vitro studies have shown superior mechanical properties for PRGF fibrin scaffolds compared to PRP. The entrapment of RBCs and leucocytes within the fibrin scaffold of the latter makes it more susceptible to degradation under inflammatory conditions (Anitua et al., 2015c).
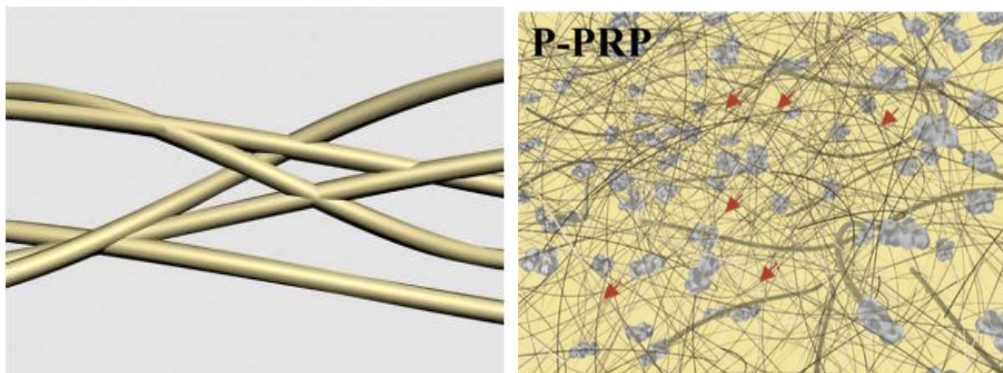
Figure 18a. (left) Theoretical computer remodelling of condensed tetramolecular or bilateral fibrin branch junctions (Dohan et al., 2006) **b.** (right) Schematic representation of matrix and architecture of pure platelet-rich plasma, or PRGF (red arrows = fibrin fibrils, grey circles = platelets) (Dohan Ehrenfest et al., 2009)

The half-life of growth factors in vivo has yet to be fully elucidated. However, in vitro studies have demonstrated an initial burst of growth factor release during the first hour after PRGF activation, with sustained release for at least 3 days. Almost

30% of this growth factor pool becomes embedded in the fibrin scaffold, and their effects exerted over a more sustained period of time. This same study showed PRGF fibrin scaffolds remained stable for at least 8 days of incubation (Anitua et al., 2016b, Del Fabbro et al., 2019).

Marx et al were the first authors to describe the physiology of specific growth factors, with their research focusing on the role of growth factors in bone regeneration during mandibular reconstructions with PCBM grafts (Marx et al., 1998). They demonstrated the presence of PDGF and TGF-β in their PRP preparation using monoclonal antibody studies. They were also able to confirm the presence of receptors to PDGF and TGF-β on cell populations in the harvested cancellous cellular marrow. Since then, there have been huge advances in the development of research in this field, to fully appreciate the therapeutic benefits of growth factors in healing and tissue regeneration.

2.2.2.3.4.1 PDGF

Platelet-derived growth factor (PDGF) is believed to be the first growth factor detected in a wound, and is responsible for initiating connective tissue healing (Marx et al., 1998). There are around 0.06ng of PDGF per one million platelets, which highlights the potency of this protein. PDGF is a powerful mitogen (stimulant of cell division) for connective tissue cells such as osteoblasts, and is also involved in macrophage activation which promotes debridement of the wound site.

2.2.2.3.4.2 TGF-β

Transforming growth factor-β (TGF-β) is one of a family of transforming growth factors, which in turn is part of a superfamily of growth and differentiating factors that includes bone morphogenetic proteins (BMPs) (Marx et al., 1998). The primary role of TGF-β is in the chemotaxis and mitogenesis of osteoprogenitor cells, and the stimulation of collagen deposition by osteoblasts. TGF-β has also been shown to inhibit osteoclast formation and bone resorption, thereby emphasising its important role in bone regeneration.

2.2.2.3.4.3 VEGF

Vascular endothelial growth factor (VEGF) is released by activated platelets and macrophages, and binds to tyrosine kinase receptors on endothelial cells. It promotes angiogenesis through endothelial cell proliferation and migration (Ferrara et al., 2003, Anitua et al., 2015a), thereby delivering nutrients and increasing blood flow to the site of injury. VEGF is upregulated by TGF-β, further enhancing the angiogenic process and recruitment of inflammatory cells to the site.

2.2.2.3.4.4 EGF

Epidermal growth factor (EGF) promotes chemotaxis and angiogenesis of endothelial cells, and mesenchymal cell mitosis. It has been shown to accelerate the healing process when concentrated at a site, as is the case with autologous

platelet concentrates. EGF receptors are found on most cell types, including fibroblasts, endothelial cells and keratinocytes (Miron and Choukroun, 2017).

2.2.2.3.4.5 IGF

Insulin-like growth factor (IGF) is a positive regulator for proliferation and differentiation of most cell types, but particularly in the late-stage differentiation and activity of osteoblasts. It is also an important regulator of apoptosis, protecting many cell types from programmed cell death by controlling survival signalling pathways (Miron and Choukroun, 2017).

2.2.2.3.4.6 HGF

Hepatocyte growth factor is a potent inducer of angiogenesis, and its receptor is abundant on endothelial cells and vascular smooth muscle cells (Lakka Klement et al., 2013). Anitua et al have demonstrated its anti-fibrotic properties at sites of tendon repair with reduced scar formation. As well as being released by platelet α-granules, its release from human tendon cells has been demonstrated with use of PRGF at sites of tendon repair.

2.2.2.3.4.7 FGF

Fibroblast growth factor binds to tyrosine kinase receptors on endothelial cells, and plays an important role in angiogenesis by promoting endothelial cell

chemotaxis and mitogenesis (Presta et al., 2005). FGF has been shown to work closely with VEGF during the angiogenic process.

2.2.2.4 Applications in different medical fields

Historically, the original application of platelet therapies was in the management of chronic non-healing leg ulcers, where wounds were covered with collagen embedded in platelet proteins (Anitua et al., 2007b). A study by Anitua et al in 2007 investigated the impact of PRGF in the management of chronic leg ulcers. Fourteen patients (7 male, 7 female) were recruited to participate in the pilot study, but only 9 patients completed the 8-week trial. Attrition arose as a result of respiratory infection, new ulcer formation and need for venous access surgery during the study period, meaning a total of only 5 experimental and 4 control patients seeing the study through to the end. The control arm involved routine debridement of the ulcer site with 0.9% normal saline warmed to room temperature, with placement of sterile dressings. The experimental arm underwent the same routine debridement, with placement of autologous PRGF fraction 2 clots over the wound, deposition of supernatant along the wound margins, and coverage of the clots with sterile dressings to secure in place. PRGF placement was repeated as necessary in the experimental arm at the weekly review visits. Mean healed skin surface area, the primary outcome measure, at 8 weeks was 72.94% (SD 22.25) in the PRGF group, and 21.48% (SD 33.56) in the control group (Anitua et al., 2007a). Baseline characteristics of the patient cohort were not disclosed, and it is possible there may have been confounding factors that resulted in the very high attrition rate. The small sample size is not adequate to fully evaluate any

appreciable treatment benefit with PRGF in the management of chronic leg ulcers, however these results are certainly promising and justify further similar research.

PRGF has a well-established role in sports medicine and orthopaedic surgery, with clinically proven benefits in functional recovery following tendon and ligament repairs (Anitua et al., 2007b). Sanchez et al looked at functional outcomes in a cohort of 6 patients who underwent surgical Achilles tendon repair with adjunctive placement of autologous PRGF prior to wound closure. When compared retrospectively to 6 matched controls who had previously undergone conventional Achilles tendon repair without PRGF, they found significant differences in the time to training activity resumption (14 ± 0.8 weeks versus 21 ± 3 weeks, p = 0.004) and the rate at which range of motion was recovered (7 ± 2 weeks versus 11 ± 3 weeks, p=0.025) (Sánchez et al., 2007). Improvements in overall tendon strength and regeneration have also been demonstrated by injection of PRP at surgical sites one-week post-operatively in similar cases (Virchenko and Aspenberg, 2006).

Ultrasound-guided intratendinous injection of PRGF has been applied successfully in the management of elbow tendinosis (Mishra and Pavelko, 2006). PRGF has also been used in patellar tendon repair surgery, and has been pioneered by the BTI research group for the arthroscopic management of articular cartilage avulsion injuries in knee joints (Sánchez et al., 2009). In response to growth factor release by PRGF, human tendon cells have been shown to proliferate and secrete VEGF and HGF, enhancing the already elevated local growth factor supply. These effects augment the angiogenic and antifibrotic benefits conferred by PRGF therapy (Anitua et al., 2007b).

Alio et al investigated the role of PRP in the management of dry eye symptoms in a case series of 386 patients. This condition is estimated to affect 4-30% of the population (Alio et al., 2017). Treatment consisted of autologous PRP eye drops applied at a dose of one drop six times per day for six weeks, with 87.5% of patients reporting improvement in their symptoms.

2.2.2.5 PRGF in oral surgery

The versatility of PRGF stems from the many permutations of preparations that can be produced from a single blood sample. Figure 19 shows the four different formulations of PRGF:

- Supernatant: this is the 'leftover' fluid after PRGF activation is complete, and can be used to treat mouth ulcers and oral lichen planus (Piñas et al., 2018) or used as eye drops, although reports are lacking in the literature in support of PRGF as a mainstay treatment modality in this respect
- Liquid PRGF – this formulation has applications specifically when used with UnicCa® implants produced by BTI. Owing to their unique feature of surface calcium coating, they undergo a process termed 'bioactivation' when immersed in activated PRGF. As the implant is surgically placed, localised growth factor release should yield superior osseointegration
- Fraction 2 clot – this platelet-rich scaffold can be placed in extraction sockets to preserve bone, maximising growth factor release at sites of application

- Fraction 1 membrane – this 'platelet-poor' component forms an elastic, dense, heamostatic fibrin and is used to promote soft tissue healing and keratinisation around implants, and during maxillary sinus lift surgery
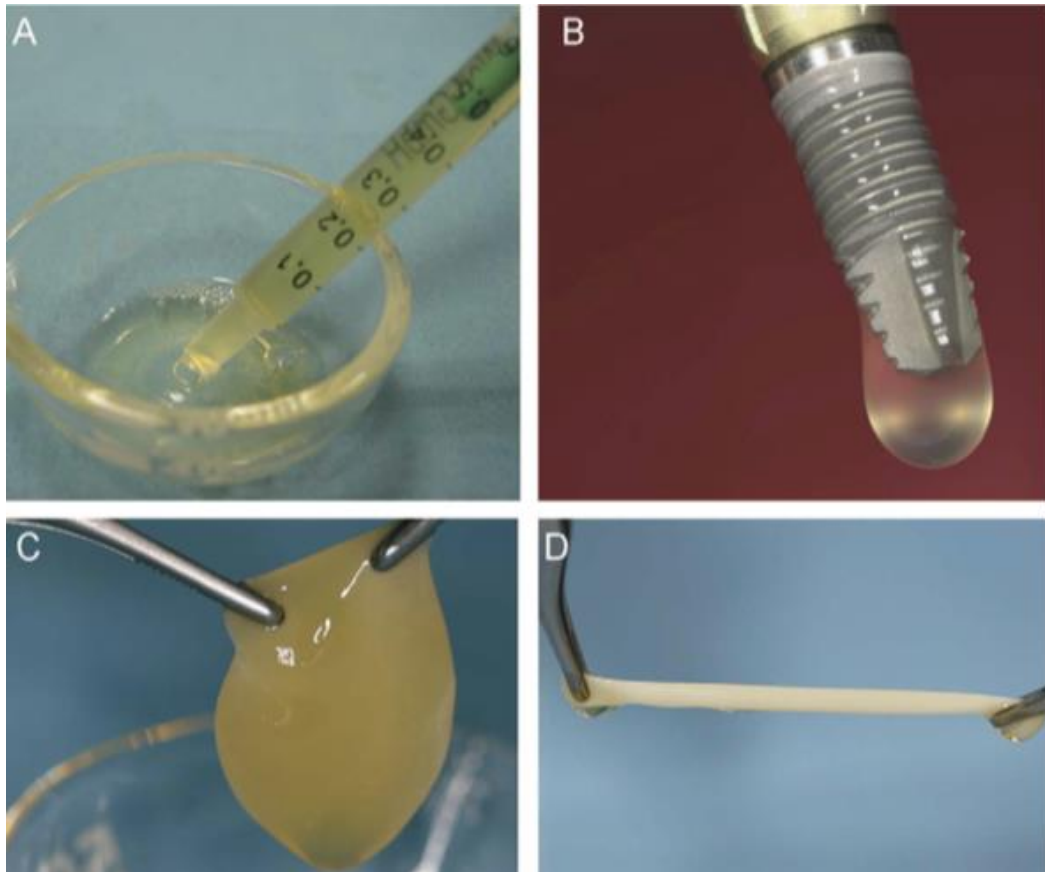
Figure 19. Various formulations of PRGF **a.** Supernatant **b.** 'Bioactivated' UnicCa® implant surface **c.** Fraction 2 'clot' **d.** Fraction 1 membrane (Anitua et al., 2007b)

PRGF was first described in the literature in the context of dental implantology. More rapid and enhanced bone healing was observed with use of PRGF at implant osteotomy sites, with the technique also producing superior soft tissue healing (Anitua, 1999). As well as the indications discussed previously, recent advances have led to clinicians combining PRGF with exogenous bone grafting material such as Bio-Oss® to improve the handling and adaptation at donor sites (Anitua et al., 2006).

One of the major challenges in dental implantology is the atrophic maxilla, and a retrospective case series published by Anitua et al suggests a promising role of PRGF in the augmentation of vertical bone height at this site. Their cohort of 26 patients underwent transcrestal sinus elevation, and PRGF plug insertion at the base of each osteotomy site prior to placement of a total of 41 implants. They observed a sustained increase in vertical bone height at three years post-operatively (Anitua et al., 2016a).

There have been at least three systematic reviews published to date on the effects of autologous platelet concentrates (APCs) on healing of extraction sockets (Del Fabbro et al., 2011, Moraschini and Barboza, 2015, Del Fabbro et al., 2017). All systematic reviews conclude a therapeutic benefit to use of APCs in extraction sockets, with reports of enhanced soft tissue healing, reduced swelling and trismus, and improvement in periodontal defects distal to the neighbouring tooth. Conclusions appear to differ with regard to post-operative pain and inflammation, with Moraschino et al reporting positive outcomes with the use of APCs, but del Fabbro et al citing inconclusive results.

A systematic review looking specifically at the effects of PRGF in post-extraction sockets was published in 2019. The authors performed a qualitative analysis of eight studies, reporting reduced postoperative pain and incidence of postoperative complications with PRGF compared to controls. There was also evidence of improved bony healing and better epithelialisation and soft tissue healing scores in the PRGF groups (Del Fabbro et al., 2019).

Medication-related osteonecrosis of the jaw (MRONJ) is a chronic and potentially debilitating condition that was first described in 2003 (Marx, 2003). It is a condition unique to patients taking bisphosphonates or antiresorptive medication for conditions such as osteoporosis, and to prevent bony fractures in patients with bony metastases. MRONJ is defined as an area of exposed bone, or bone that can be probed via an intra or extra-oral fistula for at least 8 weeks, in a patient with concurrent or previous treatment with antiresorptive or antiangiogenic medication(s), with no history of radiation therapy to the head or neck, and in the absence of metastatic disease of the jaws (Ruggiero et al., 2014). Currently, no universally agreed protocol exists for the prevention and therapeutic management of MRONJ in at-risk patients. Scoletta et al report favourable results in prevention of MRONJ in patients receiving intravenous bisphosphonates, following direct placement of autologous PRGF in extraction sockets and avoiding mucoperiosteal flaps where possible (Scoletta et al., 2013). In a retrospective case series of 32 patients undergoing treatment with intravenous bisphosphonates, Mozzati et al report total mucosal coverage with absence of exposed bone in all cases of surgical debridement and placement of PRGF, with a minimum follow-up of 48 months (Mozzati et al., 2012).

Further applications of PRGF in dentistry include (Glavina et al., 2017):

- Management of recurrent aphthous stomatitis (RAS) and refractory oral lichen planus
- Promotion of root end closure/apexification in immature teeth requiring endodontic treatment

- Treatment of gingival recession, leading to restoration of keratinised mucosa across the exposed root surface

- Arthroscopy for management of temporomandibular joint dysfunction, with conflicting results

2.2.3 Leucocyte and platelet-rich fibrin (L-PRF)

This second-generation platelet concentrate was developed in an effort to simplify the preparation process of autologous platelet concentrates, negating the need for manual fractionation and activation. Its first clinical application was in the management of a patient with persistent Lyell syndrome (toxic epidermal necrolysis) affecting the lower leg. Marked improvement in healing was observed 30 days after initial L-PRF treatment (Miron and Choukroun, 2017).

Choukroun's single-step preparation protocol involves obtaining a venous blood sample in 9 or 10mL tubes with no anticoagulant. These samples are centrifuged immediately for 12 minutes at 750g using a Process table-top PC-02 centrifuge (Nice, France) or similar. The absence of anticoagulant means platelet activation and fibrin polymerisation commence almost immediately (Dohan et al., 2006). Once centrifugation is complete, the product is a three-layered suspension, with RBC layer at the base of the tube, acellular plasma at the top and a dense fibrin clot suspended in the middle (Fig 20).
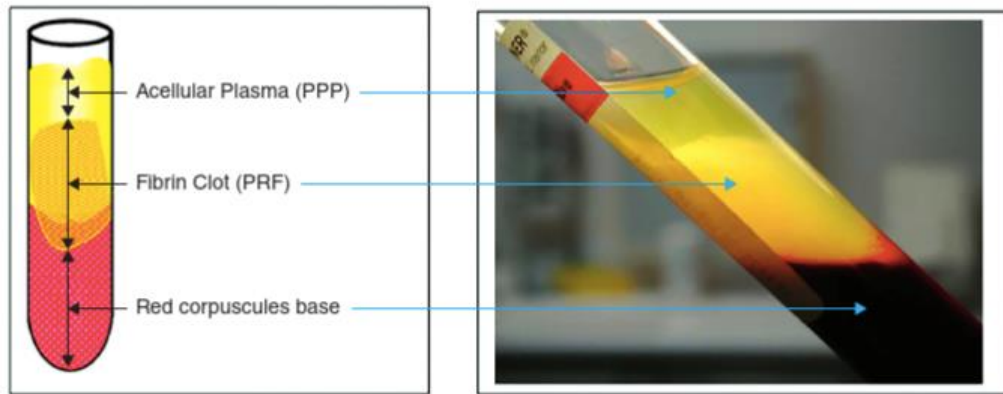
Figure 20. Leucocyte and platelet rich fibrin clot (L-PRF) after centrifugation (Miron and Choukroun, 2017)

The success of this protocol relies on quick handling of the blood samples; any delay between venepuncture and centrifugation will result in failure of the technique (Dohan et al., 2006).

The L-PRF matrix produced during this technique constitutes 50% leucocytes and 97% platelets (Pinto et al., 2018, Miron and Choukroun, 2017). To this day, the incorporation of WBCs into APCs is a matter of intense debate, with many sources advocating against this policy (Nishiyama et al., 2016, Anitua et al., 2006). In contrast to the versatility of the Endoret® system which can produce four different active formulations, the newer second-generation L-PRF protocol described in 2001 only produced one active product: the fibrin matrix. The structural properties of the L-PRF matrix differ from the Endoret® matrix. In the case of L-PRF, the alleged slower polymerisation process produces a fibrin matrix with a trimolecular polymerisation pattern, entrapping a multitude of leucocytes as well as platelets (Fig 21). In vitro studies investigating the structural properties of PRGF versus leucocyte-containing preparations have given conflicting results, with some authors reporting superior strength and tensile properties of the latter (Khorshidi

et al., 2016), while others report susceptibility of the latter to disintegration under inflammatory conditions (Anitua et al., 2015c).
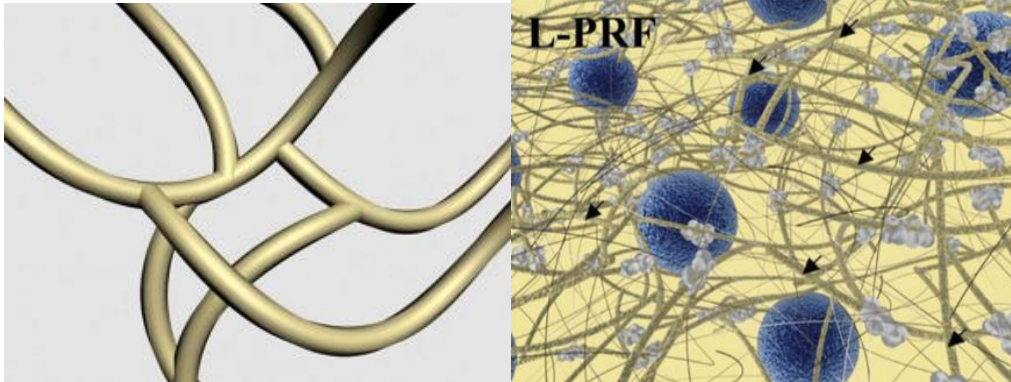
Figure 21a. (left) Theoretical computer modelling of trimolecular or equilateral fibrin branch junctions (Dohan et al., 2006) **b.** (right) Schematic representation of matrix and cell architecture of L-PRF (black arrows = thick fibrin fibrillae, grey circles = platelets, blue circles = leucocytes) (Dohan Ehrenfest et al., 2009)

2.2.4 Classifications

Some authors have proposed a classification system for autologous platelet products currently on the market (Dohan Ehrenfest et al., 2009), although this system does not appear to have garnered widespread acclaim. They propose categorisation of platelet concentrates according to three paramaters:

- Preparation kits and centrifuges used

- Content of concentrate(s) produced

- Fibrin network

Based on these three features, Dohan Ehrenfest et al devised four categories of autologous platelet products:

1. P-PRP: pure platelet-rich plasma

2. L-PRP: leucocyte and platelet-rich plasma

3. L-PRF: leucocyte and platelet-rich fibrin

4. P-PRF: pure platelet-rich fibrin

The term P-PRP is synonymous with PRGF, and would appear to be a misnomer given the number of different formulations that can be produced using the PRGF Endoret® system (Fig 19). The term cPRP or 'concentrated platelet rich plasma' has been coined as an alternative to L-PRP, in recognition of the enhanced platelet concentrations generated by two centrifugation cycles (Dohan et al., 2006, Marx et al., 1998). Further terminology has recently been added to the PRF armamentarium to reflect updates to their preparation protocols: i-PRF (injectable PRF), which refers to the plasma at the upper limit of a blood tube without anticoagulant, following centrifugation at 60g for 3 minutes (Miron et al., 2017), and A-PRF (advanced PRF) which is produced following centrifugation at 100g for 14 minutes (Kobayashi et al., 2016). Endoret® has been producing this variety of formulations for years, without any alteration of centrifugation speed or duration.

This complex array of terminologies is likely to become even more complicated with continuing advances in regenerative medicine. A simpler, more homogenous set of terminologies would seem more favourable and be more likely to achieve widespread uptake in the medical and dental communities. Sticking with original nomenclatures for simplicity, a chronological timeline of autologous platelet product development is presented in Figure 22 to summarise the evolution of this invaluable treatment modality.
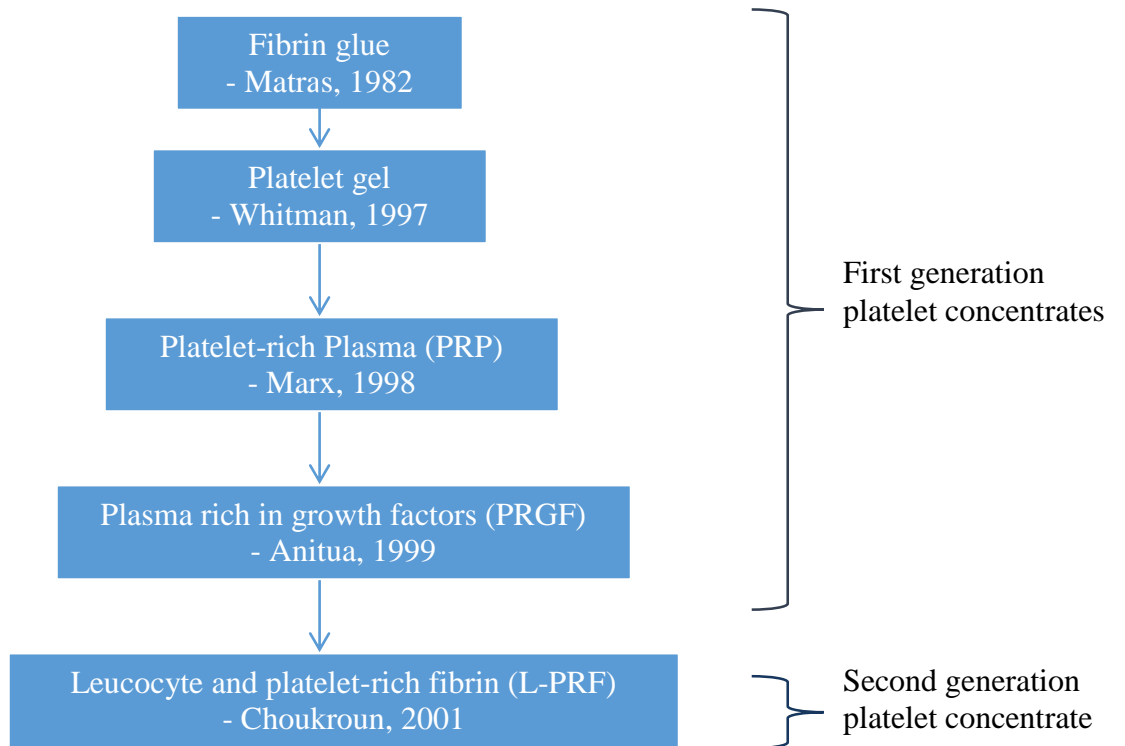
Figure 22. Flowchart presentation of timeline of autologous platelet concentrate evolution

2.3 CLINICIAN-REPORTED OUTCOME MEASURES

Despite third molar surgery accounting for the vast majority of oral surgery procedures performed worldwide (Hanna et al., 2018, Grossi et al., 2007, Jerjes et al., 2010, Ruta et al., 2000), and an ever-expanding research output on the topic, there still remains no gold standard for measurement of clinical outcomes in third molar surgery.

Oral and maxillofacial surgery (OMFS) is classified by the Royal College of Surgeons in England (RCS Eng) among the ten surgical specialties as its own distinct entity. In contrast to other surgical specialties where patients typically require in-hospital stays, many OMFS patients can be safely managed in an outpatient clinic setting, receiving treatment safely under local anaesthesia. While countries such as Australia continue to report high rates of hospitalisation for third molar surgery, this approach is often driven by external motivators such as private health insurers, rather than serving the best interests of patients (Hanna et al., 2018). With an estimated 152,000 patients undergoing third molar surgery on the National Health Service (NHS) each year in England alone (McArdle et al., 2018b), routine hospitalisation would lead to overwhelming burdens on the hospital systems in terms of bed capacity and public funding, as well as the added risks of general anaesthesia and hospital acquired infections.

The Society for Cardiothoracic Surgery (SCTS) in Great Britain and Ireland has developed a National Clinical Outcome Programme, where data are made available to the general public in keeping with the ethos outlined in their 2011

document "Maintaining patients' trust: modern medical professionalism". These data reflect surgical caseloads at each hospital on a national level, as well as operator performance. Outcomes reported include mean length of hospital stay (time from admission to discharge), re-admission rates, mortality rates and incidence of post-operative complications (SCTS, 2011). Publication of national data achieves many goals:

- Monitoring of individual hospital and operator performances

- Improvement of specialty-specific clinical governance

- Provides a reliable, transparent information source for prospective patients and family members who may need to access services

The British Association of Oral and Maxillofacial Surgeons (BAOMS) have spearheaded the Quality Outcomes in Maxillofacial Surgery (QOMS) project, led by the BAOMS Clinical Outcomes committee. National registries on free flap reconstructions following head and neck cancer surgery are published annually, as well as periodic national audit reports on subspecialty areas including temporomandibular joint (TMJ) replacement surgery, MRONJ and third molar surgery. The most recent national third molar audit report was published in January 2011, with a response rate of 38% (BAOMS, 2011). One third (32%) of patients had third molar surgery under local anaesthesia only, with a further 10% undergoing surgery with local anaesthesic and intravenous sedation. With such a low response rate, these figures are unlikely to be representative of the national status quo; improved engagement with the audit process is desirable. Clinical outcome data are still lacking however, with the national audit collecting data on third molar tooth type, indication for removal and method of anaesthesia only.

Clinician-reported outcome measures (CROMs) are the *"tangibles that are only evident to the surgeon when the patient is in the clinic"* (Ogden, 2014). CROMs, by definition, are not synonymous with postoperative complications; however, complications such as incidence of surgical site infection and postoperative haemorrhage can be considered as distinct CROMs in a clinical context. The incidence of postoperative complications following third molar surgery is known to be relatively low; in one prospective cohort study carried out by van der Sleen et al, a complication rate of 2.5% was reported among 526 patients undergoing surgical removal of a single mandibular third molar (van der Sleen et al., 2013).

Defining oral surgery-specific CROMs is far from straightforward. Inter-specialty variations in clinical outcomes precludes the transferability of existing specialty-defined CROMs; for instance, whereas mortality rate reporting would be expected for open heart surgery, the same could not be said for third molar surgery. As acknowledged by RCS Eng, there is no *"one size fits all"* approach to CROMs development. Factors such as what outcomes to measure, how to measure them, optimum timescale for reporting and risk adjustment must all be taken into consideration (RCSEng).

Third molar surgery, tonsillectomy, adenoidectomy and grommets insertion together make up the vast majority of elective surgeries undertaken in the United Kingdom (Jerjes et al., 2010). Such is the frequency with which third molar surgery is undertaken, and the predictability of its postoperative sequelae, that it is a very popular surgical model for research purposes in the clinical evaluation of various interventions and novel analgesics (Coulthard et al., 2014a).

The value of CROMs in oral surgery goes beyond service commissioning or political rhetoric. They play a key role in:

- Monitoring hospital performance and caseload, of particular relevance in publicly funded hospitals

- Evaluation of individual operator performance and case mix; this becomes particularly important where surgeons demonstrate higher than normal mortality rates in a given surgical procedure, or where specific procedure numbers are below that required to maintain clinical competence. In such instances, further training, mentorship and revalidation may be indicated.

- Education of patients regarding postoperative morbidity. Adequate information regarding procedure-specific postoperative sequelae is essential for the consent process; considering 20% of patients who undergo third molar surgery would not recommend it to others (Ogden et al., 1998), patient education is paramount.

- Standardisation of research outcomes: for researchers conducting trials in third molar surgery, having an agreed core set of robust clinical outcome measures allows objective comparisons to be made between different clinical interventions. Needless to say any agreed CROMs must be valid, reliable and acceptable to clinicians and patients.

Although there is currently no consensus agreement on a core set of oral surgery-specific CROMs, an obvious pattern of recurring outcome measures appears in the literature. The most commonly used outcome measures in third molar surgery are summarised in Table 4, and are discussed below.

Outcome measure	Method	Authors
Swelling	Three line technique I: • Po-Tr • Lab-Tr • Go-Ca	Schultze-Mosgau et al Bilginaylar et al Dar et al Bello et al
	Three line technique II: • Go-Lab • Lab-Tr • Tr-Ca	Gulsen et al
	Two line technique I: • Go-Ca • Lab-Tr	Ozgul et al
	Two line technique II: • Lab-Tr • Po-Tr	Ogundipe et al Jeyaraj et al
	Four line technique • Go-Ca • Go-Tr • Go-Po • Go-Sp	Mozzati et al
	Other: Arbitrary assessment of facial swelling, using a four-point scale from 0 'no swelling' to 3 'facial planes blurring with involvement of nasolabial folds and eyes'	Anitua et al
Mouth opening	Calipers	Jeyaraj et al Ogundipe et al Bello et al
	TRMS	Saund et al
Soft tissue healing	Landry, Turnbull and Howley healing index	Anitua et al Ritto et al Varghese et al Dutta et al
	Wound dehiscence – present/absent	Kaul et al
Bone healing	Kelly et al method (plain radiographs)	Ogundipe et al Dar et al Dutta et al

	CBCT	Jeyaraj et al Anitua et al Ritto et al
Periodontal probing depth	Williams probe	Jeyaraj et al Ritto et al Kaul et al
Dry socket	Blum's criteria Arbitrary	Dutta et al Haraji et al Eshghpour et al

Table 4. Summary of clinical outcome measures reported in the literature. Po, pogonion; Tr, tragus; Lab, labial commissure; Go, gonion; Ca, lateral canthus; Sp, nasal spine; TRMS, TheraBite® range of motion scale

2.3.1 Swelling

Pain, trismus (reduced mouth opening) and swelling are among the most common complaints by patients following third molar surgery, with a significant impact on quality of life in the postoperative period (Grossi et al., 2007). Swelling is one of the four tenets of inflammation, a normal physiological process that follows any traumatic insult such as surgery. Inflammation is the first step in the body's healing process, and is believed to exert a protective effect in the context of third molar surgery. By limiting the function of the masticatory muscles in the immediate postoperative period, a patient is encouraged to rest the surgical site and permit healing (Coulthard et al., 2014a).

A recent Cochrane review looked at nine aspects of surgical technique that might impact on postoperative swelling and other outcomes such as dry socket and sensory disturbance following third molar surgery (Bailey et al., 2020). They reviewed a total of 62 randomised controlled trials (RCTs) comparing mucoperiosteal flap technique, bone removal technique, irrigation method,

primary versus secondary wound closure, suture technique (simple interrupted versus horizontal mattress sutures), insertion of a surgical drain, lingual retractor use, autologous platelet product placement in third molar sockets (PRP and L-PRF only) and complete versus partial third molar removal (coronectomy). This review contains a number of updates to its 2014 counterpart, including an additional 27 RCTs, and the investigation of the influence of suture technique and autologous platelet products on third molar socket healing. The authors deemed the quality of available evidence to be of low or very low certainty, with over half of included trials at high or unclear risk of bias. There is weak evidence to suggest a lower incidence of dry socket at third molar sites treated with PRP/L-PRF.

While it is widely acknowledged that the unpleasant sequelae of third molar surgery can continue beyond one week post-surgery (Savin and Ogden, 1997), swelling has been shown to peak at 24-48 hours post-operatively (Savin and Ogden, 1997, Bello et al., 2011) with a significant reduction of any perceived alteration of facial appearance beyond this point. It is unfortunate that Bailey et al chose to report postoperative swelling measurements only at a time point closest to one week in their systematic review.

In the literature, swelling is one of the most commonly reported outcome measures in studies investigating the effect of PRGF, PRP and L-PRF on healing following third molar surgery (Mozzati et al., 2010, Ozgul et al., 2015, Dar et al., 2018, Gülşen and Şentürk, 2017, Ogundipe et al., 2011, Bilginaylar and Uyanik, 2016, Jeyaraj and Chakranarayan, 2018, Anitua et al., 2015a). There is notable

variation in the measurement techniques described by authors, and calls have been made for a consensus agreement on a suitable method of facial swelling measurement (Bailey et al., 2020, Coulthard et al., 2014a).

The most commonly reported method in the literature involves the sum of three linear measurements between anatomical landmarks typically used in cephalometry. Baseline measurements are taken immediately pre-operatively, and at specified intervals post-operatively according to individual study protocols. These landmarks are:

- gonion (angle of mandible)

- labial commissure (corner of mouth)

- pogonion (most prominent point on the midline of the chin)

- tragus (most posterior point on the midline of the fleshy cartilage)

- lateral canthus (corner of eye)

Three lines are drawn to connect tragus to labial commissure, tragus to pogonion and gonion to lateral canthus, and measurements are recorded in millimetres using a flexible ruler (Fig 23). The sum of these three measurements is used to calculate swelling as a percentage using the following formula (Ogundipe et al., 2011):

$$S_{post\text{-}op} - S_{pre\text{-}op} / S_{pre\text{-}op} \times 100$$

This, along with other less commonly reported measurement methods, are summarised in Table 4.
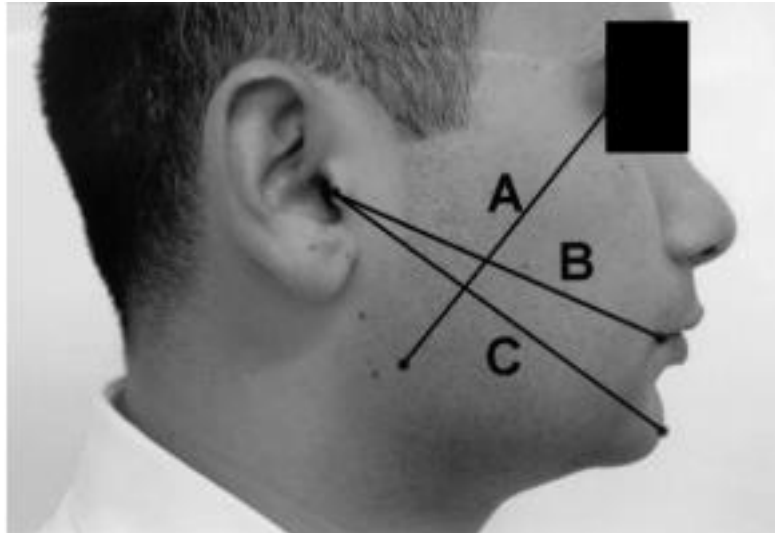
Figure 23. Anatomical landmarks used in the measurement of facial swelling (Ustün et al., 2003) Line A = gonion to lateral canthus; Line B = tragus to labial commissure; Line C = tragus to pogonion

There is a paucity of well-conducted randomised controlled trials in the literature looking specifically at the use of PRGF in third molar surgery. Mozzati et al (2010) undertook clinical and biological soft tissue analysis on a group of sixteen patients aged 18-35 years requiring surgical removal of bilateral mandibular third molars, using a split-mouth study design. Their primary aim was to elucidate the various growth factors and cytokines released at the control surgical third molar sites and compare to the experimental PRGF-treated third molar sites, while also investigating the clinical outcome of facial swelling (Mozzati et al., 2010). They adopted a four-line technique for facial swelling measurement (see Table 4), citing a method previously described elsewhere (Neupert et al., 1992). A daily facial swelling measurement was recorded for each side of the face by the patients themselves (with the exception of days 1 and 7 postoperatively). Given the complexity of the four-line technique described by the authors, it would seem unlikely that the values recorded were reliable, and the statistical significance reported by the authors should therefore be appreciated with caution.

Another RCT looking at facial swelling as an outcome measure in PRGF-treated extraction sockets has been published by Anitua et al (2015). Theirs is a parallel group design, with a total of 60 patients undergoing removal of a single molar tooth. The authors describe a rather elusive method of facial swelling measurement, labelling it as 'inflammation'. They score 0 for patients with no swelling, 1 for patients with slight swelling and hardness 'without facial planes blurring' and so forth to a maximum score of 3 for 'facial planes blurring with involvement of nasolabial folds and eyes' (Anitua et al., 2015a). Such a method has not been found elsewhere in the literature. This study excluded impacted third molar teeth, and is therefore discussed here purely for completeness.

Certain problems arise when considering the reproducibility of facial swelling measurement as a gold standard CROM; firstly, facial swelling will vary depending on the time of day at which it is measured, and more specifically on the length of time during which a patient has been ambulatory. A measurement taken at 9am will inevitably differ from one taken at 5pm, as gravitational forces from general day-to-day activity will reduce inflammatory collections in the facial region and therefore influence the extent of facial swelling. Patient positioning in the dental chair will also have an impact; a patient lying supine will invariably have a higher facial swelling measurement than if (s)he were sitting upright in the chair.

The lack of a standardised measurement technique and glaring reproducibility challenges render facial swelling an unfavourable CROM for the purposes of third molar surgery in its current format.

2.3.2 Mouth opening

Normal adult mouth opening is generally around 40-50mm, and a diagnosis of trismus typically implies less than 30mm mouth opening (McGrath et al., 2003c). Trismus is a common complication of surgical procedures performed within the oral cavity, such as third molar removal (Saund et al., 2012). It is also seen in patients with disorders of the temporomandibular joint (TMJ), mandibular trauma, fibrotic conditions of the oral mucosa such as oral submucous fibrosis (OSF) arising as a result of long-term betel chewing, and trismus is often an indicator of pathology such as spreading dental infection involving the fascial spaces or in cases of oropharyngeal malignancy. Trismus is, in fact, a marker of many disease processes affecting the maxillofacial region.

Monitoring of patients' mouth opening is commonly undertaken as a measure of postoperative morbidity or as an outcome measure following therapeutic interventions (Saund et al., 2012). Maximum mouth opening, also referred to as interincisal distance, is the measurement in millimetres between the incisal edges of ipsilateral maxillary and mandibular central incisors (Jeyaraj and Chakranarayan, 2018, Ogundipe et al., 2011, Bello et al., 2011). Some authors advocate more specific points of reference, such as the mesio-incisal corners of the upper and lower right central incisors (Bilginaylar and Uyanik, 2016, Ustün et al., 2003), although the incisal edge is generally the preferred and more widely accepted landmark for measurements. Mouth opening measurement relies on the presence of intact incisor teeth, or at least the presence of prosthetic teeth (Saund et al., 2012) and may not be applicable in certain instances such as hypodontia or

oligodontia (congenitally absent teeth) or where a patient has previously had the incisor teeth extracted and not replaced.

Measurement of mouth opening is typically achieved using a flexible disposable ruler or sterilisable steel ruler, or with use of calipers (Jeyaraj and Chakranarayan, 2018, Bello et al., 2011, Ogundipe et al., 2011). Most researchers investigating mouth opening as an outcome measure following third molar surgery will record these values in the clinic at specified time points which tend to vary according to study protocols. In many instances, there is a requirement for patients to return to the clinic up to three times during the immediate postoperative week (Bilginaylar and Uyanik, 2016, Ogundipe et al., 2011, Bello et al., 2011). This scenario is not always practicable, particularly in instances where patients reside a considerable distance away from the clinic, and may have financial implications for patients needing to take time away from work in order to attend.

Saund et al (2012) investigated the validity and reliability of the TheraBite® range of motion scale (TRMS) for self-measurement of mouth opening among a cohort of 80 patients undergoing third molar surgery (Fig 24). Each patient was instructed on the use of the instrument, and was asked to record a measurement each evening for seven days following surgery. They also attended the clinic on days 2 and 7 postoperatively. The authors demonstrated excellent reliability of patients' measurements with an intraclass correlation coefficient of 0.92. Patients' and clinicians' measurements were also highly correlated (rho=0.82, p<0.0001). The TRMS may have a significant role to play in research studies where frequent

mouth opening measurements are required to monitor disease progression or response to an intervention (Saund et al., 2012).

Figure 24. TheraBite® range of motion scale
https://www.craniorehab.com/therabite-atos-rom-scales.html

To date, no researchers have explored mouth opening as an outcome measure following PRGF placement in third molar sockets. Interestingly, none of the four studies included in the 2020 Cochrane Review by Bailey et al exploring PRP/L-PRF use in mandibular third molar sockets, reported trismus as an outcome measure (Gülşen and Şentürk, 2017, Ozgul et al., 2015, Dutta et al., 2015, Eshghpour et al., 2014).

It is, however, a long-established fact that trismus is an inevitable sequela of third molar surgery. Saund et al (2012) reported a 33% reduction in mouth opening two days following removal of a single impacted mandibular third molar in their cohort of 80 patients (mean preoperative measurement 47.8mm; mean measurement two days postoperatively 31.9mm). Some authors have demonstrated a positive correlation between trismus and analgesic consumption in the postoperative period, which in turn negatively impacts on patients' quality of life (Grossi et al.,

2007). The same authors have also revealed a greater incidence of trismus in those aged 23years and over following third molar surgery.

2.3.3 Soft tissue healing

In periodontology, soft tissue healing assessment is widely undertaken to monitor healing following periodontal surgery. A healing index devised by Landry et al (1988) for this purpose, has been widely adopted by the oral surgery community for use in grading extraction socket healing (Table 5). Healing is scored on a scale of 1 (very poor healing) to 5 (excellent healing) by visual inspection of the surgical site.

Healing index	Criteria
Very poor 1	▪ Tissue colour: >50% of gingivae red in colour ▪ Response to palpation: bleeding ▪ Granulation tissue: present ▪ Incision margin: not epithelialised, with loss of epithelium beyond margins ▪ Suppuration: present
Poor 2	▪ Tissue colour: >50% of gingivae red in colour ▪ Response to palpation: bleeding ▪ Granulation tissue: present ▪ Incision margin: not epithelialised with connective tissue exposed
Good 3	▪ Tissue colour: <50% of gingivae red ▪ Response to palpation: no bleeding ▪ Granulation tissue: none ▪ Incision margin: no connective tissue exposed
Very Good 4	▪ Tissue colour: <25% of gingivae red ▪ Response to palpation: no bleeding ▪ Granulation tissue: none ▪ Incision margin: no connective tissue exposed
Excellent 5	▪ Tissue colour: pink gingivae ▪ Response to palpation: no bleeding ▪ Granulation tissue: none ▪ Incision margin: no connective tissue exposed

Table 5. Landry, Turnbull and Howley soft tissue healing index (Landry et al., 1998)

Several studies looking at the use of autologous platelet concentrates in mandibular third molar sockets use this index as either a primary or secondary outcome measure following third molar removal (Varghese et al., 2017, Ritto et al., 2019, Dutta et al., 2015, Kaul et al., 2012). Only one RCT looking at PRGF at non-third molar sites has been found to report soft tissue healing as an outcome measure (Anitua et al., 2015a). This parallel group design RCT (n=60) reported significantly higher soft tissue healing scores in the PRGF group on days 3, 7 and 15 postoperatively.

Similar studies using L-PRF have yielded conflicting results, with some reports of superior soft tissue healing at L-PRF treated socket sites compared to controls (Varghese et al, 2017) while other sources fail to show any significant difference (Ritto et al, 2019). A further study comparing PRP-treated mandibular third molar sockets and control sites in a cohort of 60 patients in a parallel group design showed statistically significantly better soft tissue healing in the PRP group on days 3 (p<0.002), 7 (p<0.001) and 14 (p<0.003) postoperatively. Kaul et al (2012), rather than adopt the Landry et al healing index, report on wound dehiscence as either present or absent in their split-mouth study of 25 patients undergoing bilateral mandibular third molar removal with PRP placement on one side. They reported an 8% wound dehiscence rate at PRP-treated sites, compared to 92% at control sites.

The Landry et al healing index has been modified for application in a dental extraction context (Pippi et al., 2015), to include the following seven clinical parameters: gingival colour, granulation tissue, degree of epithelialisation,

swelling, bleeding, pain and suppuration. Each parameter is scored 0 (bad) or 1 (good), with the highest score of 7 indicating excellent healing. This modified index is described in Table 6. Its simplicity renders it favourable for use in clinical trials.

Parameters		Score 0	Score 1
Inspection	Gingival colour	Totally/partially red	Pink
	Granulation tissue	Present	Absent
	Epithelialisation	Partial	Complete
	Swelling	Present	Absent
Palpation	Bleeding	Present	Absent
	Suppuration	Present	Absent
	Tenderness	Present	Absent

Table 6. Modified Landry et al healing index (Pippi et al., 2015)

There is currently no consensus as to the optimal time to perform soft tissue assessment post-surgery. Some authors advocate evaluation of soft tissue healing soon after surgery, to fully elucidate any potential effects of platelet products (del Fabbro et al, 2011). A systematic review looking at the influence of PRGF on alveolar socket healing has shown superior epithelialisation and increased thickness of keratinised tissue at PRGF-treated sites, particularly in the first two weeks postoperatively. By the 21st postoperative day, there is very little difference in soft tissue healing scores between groups (del Fabbro, 2015). These findings would certainly be in keeping with the physiology of growth factor release by platelets, discussed in section 2.2.2.3.4.

2.3.4 Bone healing

Monitoring of alveolar bone healing is a priority in many circumstances such as dentoalveolar trauma in children and adolescents in whom growth may not yet

be complete, and where early loss of premolar and molar teeth may lead to space loss and occlusal disturbances long term, if left untreated. In such instances, timing is paramount, and rehabilitation of edentulous spaces with dental implants remains the gold standard for long-term success. Assessment of alveolar bone height, ridge width, cortication and proximity to anatomical structures such as the maxillary antrum and inferior alveolar and mental nerves, is a fundamental part of dental implant treatment planning. Alveolar bone healing assessment becomes less important at second and third molar sites, where implant placement is almost unheard of, but may be indicated where coronectomy has been performed, or where there is suspicion of inferior alveolar nerve injury.

Numerous researchers have explored the effects of APCs on alveolar bone healing using either serial plain radiographs (Varghese et al., 2017, Kaul et al., 2012, Dutta et al., 2015, Ogundipe et al., 2011, Jeyaraj and Chakranarayan, 2018) or CBCT slices (Anitua et al., 2015a, Ritto et al., 2019).

In the case of plain radiographs, Kelly's method appears to be the most commonly cited approach to alveolar bone healing assessment (Ogundipe et al., 2011, Dar et al., 2018, Dutta et al., 2015, Jeyaraj and Chakranarayan, 2018). This method grades three parameters on a 5-point scale (-2 to +2), namely lamina dura, overall density and trabecular pattern (Kelly et al., 1980). The baseline preoperative radiograph is given a score of 0; a score of -1 to +1 indicates moderate deviation from normal, while a score of -2 to +2 indicates significant deviation (see Table 7). In one study, patients returned 8 weeks postoperatively for radiographic assessment (Jeyaraj and Chakranarayan, 2018), in another they returned at 4 and

12 weeks postoperatively (Dar et al., 2018), and in two studies patients returned on three occasions up to 4 months postoperatively (Dutta et al., 2015, Ogundipe et al., 2011). There is notable disparity in the time points at which radiographic assessment is deemed appropriate. Radiation exposure should be kept to a minimum in line with the Ionising Radiation Regulations (2017); serial postoperative radiographs undertaken without due clinical justification should be avoided.

Lamina dura
+2 Lamina dura essentially absent, may be present in isolated areas
+1 Lamina dura substantially thinned, missing in some areas
 0 Within normal limits
−1 Portions of lamina dura thickened, milder degrees
−2 Entire lamina dura substantially thickened
 0 Within normal limits
Overall density
+2 Severe increase in radiographic density
+1 Mild to moderate increase in radiographic density
 0 Within normal limits
−1 Mild to moderate decrease in radiographic density
−2 Severe decrease in radiographic density
Trabecular pattern
+2 All trabeculae substantially coarser
+1 Some coarser trabeculae; milder degrees
 0 Within normal limits
−1 Delicate finely meshed trabeculations
−2 Granular, nearly homogenous patterns; individual trabeculations essentially absent

Table 7. Modified Kelly et al bone healing index (Ogundipe et al., 2011)

Ritto et al (2019) performed bone healing analysis using baseline preoperative CBCT and a single postoperative CBCT of the site(s) of interest at 3 months post-surgery. In their split-mouth study of L-PRF placement in a single third molar socket among 17 patients, they demonstrated excellent reproducibility of pre- and post-operative bone density measurements with a reported intraclass correlation

coefficient >0.98 using ITK-SNAP 3.0 software (Cognita, Philadelphia, PA, USA).

Anitua et al (2015) in their PRGF investigative RCT compared the Hounsfield value of extraction sites at baseline (immediately post-extraction) and 10-12 weeks postoperatively, using axial slices of a CBCT scan (Fig 25). They demonstrated accelerated bone deposition at PRGF-treated extraction sites compared to controls.
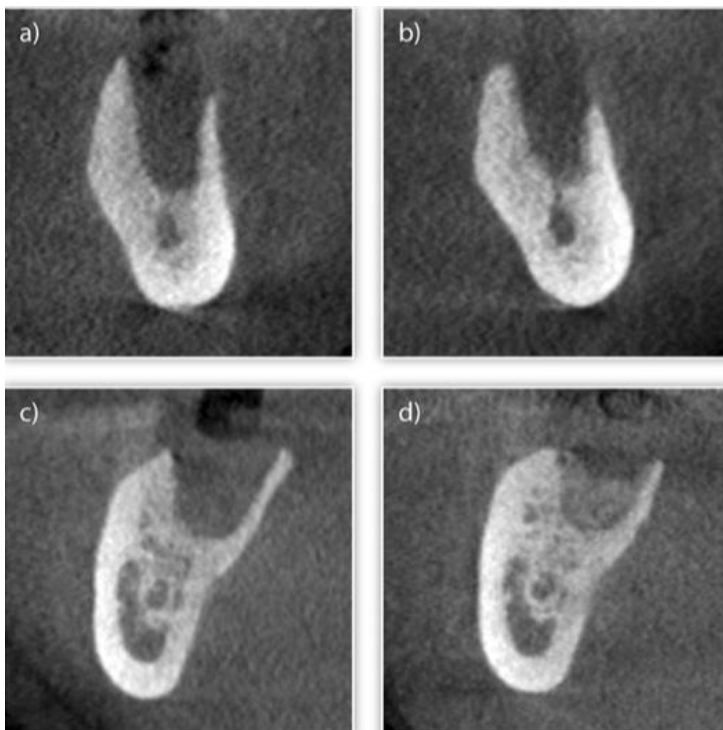
Figure 25. Axial CBCT slices showing status of third molar extractions sites at baseline (immediately post-extraction) and after 10-12 weeks of healing **a.** control group at baseline and **b.** after 10-12 weeks **c.** experimental group at baseline and **d.** after 10-12 weeks (Anitua et al., 2015a)

Some would argue that the most accurate method for evaluating bone healing is to access the surgical site following a period of healing, and harvest a specimen of bone for histological examination (Varghese et al., 2017). To date, there has been only one study where histological reporting of the healing extraction site has been undertaken in PRGF-treated, and control, molar sites (Anitua et al., 2015a). In

their cohort of 60 patients, bone biopsy was performed in 21 patients from the experimental arm and in 5 control patients. These specimens were collected in 4% buffered formalin and processed without decalcification. Final histological sections were prepared and stained using haematoxylin-eosin and May-Grunwald-Giemsa stains (Fig 26). They demonstrated 63.1% mean bone deposition in the PRGF group, compared to 35.6% mean bone deposition in the control group.
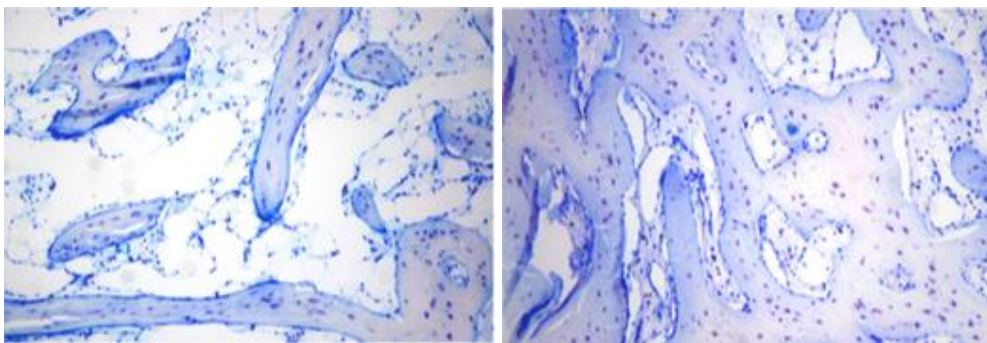
Figure 26. Histological slides showing post-operative bone deposition at control site (left) and PRGF site (right). Note smaller lacunae and greater bone density on the right (Anitua et al., 2015a)

The clinical value of establishing alveolar bone levels at third molar extraction sites is questionable, and would arguably be better reserved for premolar or first molar sites planned to receive dental implants.

Use of long cone intraoral periapical radiographs (IOPA) for assessment of bone healing has a high potential for error. The reproducibility of such images cannot be guaranteed, and even slight differences in angulation of the film-holding instrument can have a huge impact on the quality and radio-opacity/lucency of the resulting image. Many institutions will also have several radiographers employed to take radiographs, and with inter-individual variation in technique and experience.

Finally, and most pertinently, the use of ionising radiation without due clinical justification, has serious ethical and moral implications. It is certainly at odds with our Hippocratic motto: 'primum non nocere' ('first do no harm').

2.3.5 Periodontal probing depth

As discussed in section 2.1.3.3, periodontitis is one of the most common indications for third molar surgery, and is particularly prevalent in cases of horizontal third molar impaction (McArdle et al., 2018a). Some authors report bony periodontal defects along the distal surface of adjacent second molar teeth in up to 44% of cases where the M3M has been removed (Kugelberg et al., 1985).

Measurement of periodontal pocket depths is one of the first techniques learned by aspiring dentists during their undergraduate training years, and is widely used in epidemiological studies due to its reproducibility. The Williams periodontal probe is commonly used for this purpose, with the periodontal pocket depth corresponding to the distance between the free gingival margin and the point of resistance at the base of the pocket (Fig 27).

Figure 27. Williams periodontal probe www.lm-dental.com

There are some published studies that investigate the periodontal health at third molar sites with application of L-PRF/PRP (Kaul et al., 2012, Ritto et al., 2019, Jeyaraj and Chakranarayan, 2018), but none using PRGF could be found. Kaul et al (2012) in their split-mouth study of 25 patients, calculated their PPD measurements as the average of three readings – mid-distal, distobuccal and distolingual of the adjacent second molar tooth. This process was undertaken pre-operatively, and at five different post-operative time points, the final at 6 months post-operatively. The differences in PPD between control and PRP-treated sockets was found to be statistically significant at 3 and 6 months postoperatively. Ritto et al (2019) recorded PPD at baseline and 3 months postoperatively in their cohort of 17 patients undergoing bilateral M3M removal, but failed to show a statistically significant difference in probing depths between control and L-PRF treated sites. Jeyaraj et al (2019) in their parallel group RCT of 60 patients, recorded PPD at baseline and 8 weeks postoperatively, and reported reduced PPD at L-PRF treated sites. Their measurement method involved a single PPD reading at the distobuccal aspect of the adjacent second molar tooth.

The simplicity, validity and reliability of periodontal probing depth measurement lend favour to its use as a CROM in third molar surgery. One obvious disadvantage is the need for patients to return to the clinic several weeks or months following surgery, which brings with it an increased potential for attrition bias.

2.3.6 Dry socket

Dry socket, or alveolar osteitis (AO), is a relatively common complication following dental extraction (Fig 28), with a reported incidence of up to 37% (Sharif et al., 2014). Locally increased fibrinolytic activity within the extraction socket is believed to be significant in the pathogenesis of AO, with anaerobic bacterial activity also playing a role (Blum, 2002). Risk factors such as surgical trauma, smoking habit and poor compliance with postoperative care can increase the risk of developing AO. Operator inexperience has been cited by some sources as a contributor to development of AO postoperatively (Jerjes et al., 2010) with other authors failing to corroborate this finding (Grossi et al., 2007).

Figure 28a. (left) lower right molar socket at day 3 post-extraction. The blood clot has been lysed by macrophages and migration of fibroblasts into the clot lay down granulation tissue providing a framework for further healing **b.** (right) Clinical presentation of dry socket at upper left first molar site, showing exposed necrotic bone and absence of granulation tissue (Sharif et al., 2014)

AO demonstrates a distinct predilection for mandibular molar sites (Sharif et al., 2014). Such is the perceived associated risk in the case of third molars, that Bailey et al (2020) cite development of AO as one of their primary outcome measures in their recent Cochrane review. In the context of APCs and their impact on healing following M3M surgery, however, very few RCTs appear to report AO as a primary or secondary outcome measure. One RCT was found in the literature to

investigate AO as a reported outcome measure following PRGF placement in M3M sockets (Haraji et al., 2012). In fact, Bailey et al (2020) were unable to draw any conclusions regarding the effect of flap design, suturing technique, primary versus secondary wound closure, irrigation method, bone removal technique, partial versus complete M3M removal, surgical drain placement and lingual retractor use, on the incidence of AO following M3M surgery. They found low-quality evidence supporting a reduced incidence of AO with use of PRP/L-PRF.

A working definition of alveolar osteitis has been proposed as *"postoperative pain in and around the extraction site, which increases in severity at any time between 1 and 3 days after the extraction accompanied by a partially or totally disintegrated blood clot within the alveolar socket with or without halitosis"* (Blum, 2002). The pain associated with AO tends to be refractory to common analgesics, and patients will invariably need to return to the dental surgery for management with local measures; irrigation of the socket with normal saline or chlorhexidine mouthwash and placement of an obtundent dressing such as Alveogyl® at the extraction site (Blum, 2002). The morbidity associated with development of AO, as well as the financial and social implications of returning to the dental surgery and missing time from work and other activities, respectively, can have a significant impact on quality of life.

One single-centre retrospective analysis of 904 third molar extractions in 499 patients showed a significantly lower incidence of AO in patients whose sockets were treated with PRP (Rutkowski et al., 2007). The authors did not report any specific diagnostic criteria with respect to AO. Dutta et al (2015) in their parallel

group study of 60 patients undergoing removal of a single M3M with or without PRP, specified Blum's criteria as the benchmark for a diagnosis of AO. These criteria were based on Blum's earlier definition:

1. Postoperative pain in and around the extraction site

2. Partially or totally disintegrated blood clot

3. With/out halitosis

4. With/out necrotic debris

5. Denuded socket

6. Exudate or pus in socket

The authors, however, failed to discuss the outcome in their results so unfortunately, no conclusions can be drawn in this instance. In a further split-mouth trial of 78 patients undergoing bilateral M3M removal, AO was over twice as likely to affect control sites compared to sites treated with L-PRF (Eshghpour et al., 2014). Similarly, Haraji et al (2012) demonstrated a statistically significant difference in the incidence of AO at PRGF-treated third molar sites compared to controls in their split-mouth RCT of 40 patients, favouring PRGF use in third molar surgery. Their cohort was split between maxillary (n=20) and mandibular (n=20) third molar removal, a factor that will impact on the results due to the higher propensity for AO development at mandibular sites.

Despite the frequency with which dry socket is encountered in clinical practice, there is a paucity of reports on AO as a clinical outcome measure in M3M studies. Efforts should be made to standardise AO as a CROM in future M3M research.

2.3.7 Sensory disturbance

Inferior alveolar and lingual nerve injuries have been discussed previously in section 2.1.6.1. Sensory disturbance is widely accepted as being one of the most serious risks associated with M3M surgery, and in many instances, surgeons advise against complete removal of the tooth, instead recommending coronectomy or partial M3M removal to mitigate this risk. Alteration or loss of sensation to the inferior alveolar and lingual branches of the mandibular nerve can have grave consequences for patient quality of life, and even greater medicolegal ramifications for oral surgeons (Renton, 2010).

Sensory disturbance is considered temporary where normal sensation recovers within four to six months (Coulthard et al., 2014a, Bailey et al., 2020), and permanent where disturbance continues beyond six months. The relatively low incidence of iatrogenic trigeminal nerve injury during M3M surgery would suggest that sensory disturbance reporting is of limited value in small scale randomised clinical trials, where very few if any cases of nerve injury may be reported. In spite of this, permanent and temporary sensory disturbance of lingual and IAN distributions of the trigeminal nerve accounted for two of the five primary outcome measures reported in the recent Cochrane review of mandibular third molar surgical technique (Bailey et al., 2020).

The need for long-term follow-up of iatrogenic trigeminal nerve injuries is another barrier to routine use of sensory disturbance as a CROM in third molar surgery. Some authors recommend review of affected patients at 1 week, 1 month, 6 months

and 1 year postoperatively (Poort et al., 2009) although this is likely to vary between institutions. Methods for assessing nerve injuries include light touch, pin prick test, two-point discrimination and thermal test (Fig 29), and mapping the affected area of the skin for clinical monitoring. Onward referral to tertiary specialist centres is generally advised where no improvement is seen within three months of surgery (Renton, 2013).
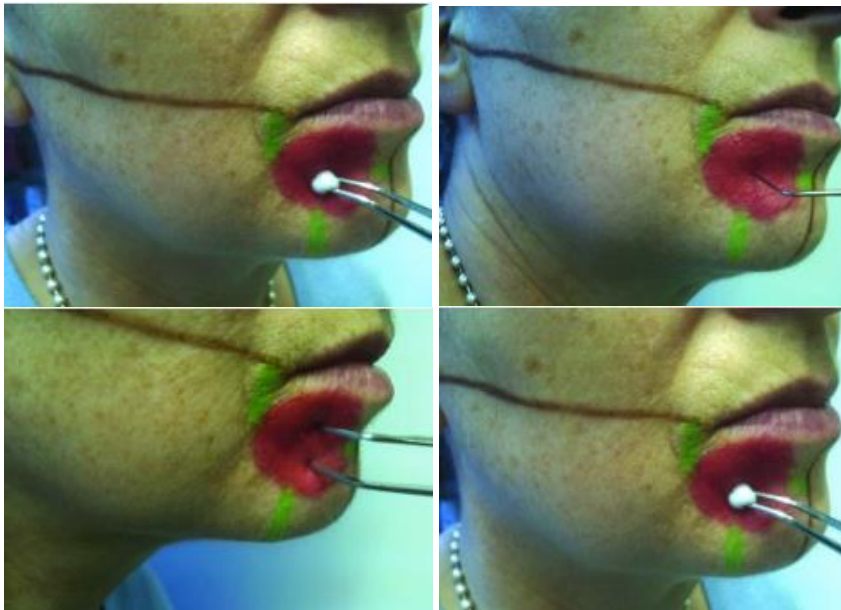
Figure 29 (a-d). (clockwise from top left) **a.** Light touch discrimination **b.** Pin prick test using dental probe **c.** Thermal test using cooled/heated cotton pellet **d.** Two-point discrimination using dental tweezers (Mahon and Stassen, 2014)

It would seem reasonable that sensory disturbance be reserved as a CROM in large-scale, multicentre trials where reporting is likely to better reflect reality.

2.3.8 CROMs selection

Based on the above critique of outcome measure reporting in the third molar surgery literature, a pragmatic selection of CROMs appropriate to the research topic of this thesis was performed at the outset:

- Mouth opening

- Soft tissue healing (using modified index described by Pippi et al)

- Periodontal probing depth

- Alveolar osteitis

Mouth opening is the preferred CROM in this instance due to its reproducibility and cost-effectiveness. On reflection, investment in a stock of TheraBite® devices would be prudent for future studies incorporating mouth opening as an outcome measure in light of its proven validity and reliability. It would also facilitate additional data collection, enabling patients to measure and record their own mouth opening without attending the clinic, which might also be of relevance to other departments undertaking research within the dental hospital. The discriminatory prowess of the soft tissue healing index in an oral surgery context may prove questionable; it was chosen in this instance due to its applicability in determining the rapidity of the healing process, which is of particular significance in evaluating the potential healing effects of PRGF (Del Fabbro et al., 2011). In retrospect, periodontal probing depth evaluation is better suited to a more longitudinal follow-up period than the one-week follow-up specified in this protocol; nevertheless, clinical information regarding periodontal status at pre-operative third molar sites is of value to the oral surgery profession, particularly in view of the increasing doubt being cast over the relevance of current NICE guidance. Finally, alveolar osteitis reporting is also included due to the frequency with which it is seen following dental extractions. Any intervention that may reduce the risk of developing AO postoperatively is likely to garner attention among the profession.

2.4 PATIENT-REPORTED OUTCOME MEASURES

The role of the patient in establishing disease chronology and meaningful outcomes of surgical and pharmacological interventions, is now widely considered crucial in evaluating the quality of healthcare services, and should be seen as complementary to objective clinical outcome measures. This shift away from the traditional disease-centred approach to healthcare provision towards a more patient-centred ethos, has been lauded by some as transformative to healthcare services, and has prompted widespread implementation of patient-reported outcome measures (PROMs) in Sweden, England and the United States (Black, 2013).

A PROM is a standardised instrument (typically a questionnaire) that enables patients to self-evaluate one or more aspects of their health (Devlin and Appleby, 2010), such as functional status and health-related quality of life (Gerrard et al., 2017). PROMs were originally developed for use in research, but have since been adopted by medical professionals to aid clinical decision-making, and to assess outcomes of treatment provision (Black, 2013). Policy makers may also look to PROMs data to inform funding decisions, prioritising patient groups deemed most in need of resources.

PROMs are distinct from patient-reported experience measures (PREMs), which focus more on qualitative aspects of the humanity of care such as being treated with dignity and being involved in the decision-making process (Black, 2013,

Gerrard et al., 2017). PREMs play an important role in audit, quality improvement and service evaluation projects.

There are many challenges when incorporating PROMs into everyday clinical practice, not least the financial implications of collecting, analysing and presenting PROMs data and the additional time required to do so (Black, 2013). Concerns have also been voiced about the potential for political misuse of PROMs data leading to crude rationing of healthcare services (Black, 2013). Deciding which patient groups are most deserving of interventions should not be based solely on quality of life reports, which may fail to capture significant aspects of a patient's health status. The "disability paradox", which describes a disparity in quality of life rating among those with severe disease versus those with mild ill-health, makes it difficult to directly compare patient groups with different disease entities for the purpose of resource allocation (Higginson and Carr, 2001).

NHS England mandated the collection and analysis of PROMs data in April 2009 for a number of elective surgical procedures including hip replacement, knee replacement, inguinal hernia repair and varicose vein surgery, with four key aims in mind (NHS):

- Assessment of individual provider performance

- Research on optimal treatment options

- Establishment of baseline pre-operative health status of patients

- Reduction of health inequalities

The collection of PROMs on a national scale produces worthwhile effectiveness data, which in turn helps to inform clinical decision-making. In the case of one healthcare trust, where health gains for knee and hip replacement surgery were found to fall well below the national average during the period 2009-2011, the decision to switch implant brand led to a significant improvement in subsequent health gains data. The inception of the mandatory national audit coincided with a pre-existing voluntary audit that had been in progress since 2008 as part of the Patient Outcomes in Surgery (POiS) initiative. The latter was introduced to compare the case-mix adjusted patient-reported outcomes and complication rates of elective surgery undertaken by privately funded Independent Sector Treatment Centre (ISTC) providers and publicly-funded NHS providers, in response to suggestions that ISTCs may have worse outcomes, and may be engaging in selective siphoning of simpler cases that are essential for surgical training in NHS institutions. The audit report released by RCS Eng in October 2011 reported slightly better outcomes in favour of ISTCs (Chard et al., 2011).

RCS Eng now asks all providers of cosmetic surgery to routinely collect baseline and postoperative PROMs data for patients undergoing abdominoplasty, rhinoplasty, blepharoplasty, augmentation mammoplasty, liposuction and rhytidectomy (facelift). Collection of health-related QoL data for this patient cohort is essential to truly appreciate the risk-benefit analysis of cosmetic surgery.

Use of PROMs in oral surgery is not a novel concept. The first preliminary reports into the impact of third molar surgery on quality of life date back over twenty years, where researchers collected data from 29 patients on days 1 and 7

postoperatively (Savin and Ogden, 1997). The results of this study helped inform the design of a disease-specific third molar quality of life instrument, the postoperative symptom severity (PoSSe) scale, which to this day remains the only disease-specific PROM of its kind with proven validity and reliability.

The NHS Guide for Commissioning Oral Surgery and Oral Medicine (2015) was published with a view to assessment of four key quality and outcome areas: access, communication, value for money and clinical care. The document outlines a series of seven PREMs for use in oral surgery and oral medicine, which focus on clear communication in plain language, adequate pain control and anxiety management, and timely instructions with point of contact details in the event of postoperative complications or adverse effects of prescribed medications (NHS England Chief Dental Officer Team, 2015). The suitability of these PREMs in their current format has been called into question by some authors who recommend replacing the current three-point 'agree/disagree/not sure' scale with a five-point Likert scale to collect a broader range of data. This is preferred for better detection of variability in provider performance and patient satisfaction (Gerrard et al., 2017).

The core oral surgery PROMs outlined in the NHS Commissioning Guide (Table 8) are reportedly based on NHS Classifications OPSC-4 and the WHO ICD10 Classification of Disease, since superseded by ICD11 (Organisation, 2010). To date, only two studies have investigated the use of these core PROMs in an oral surgery patient cohort, one each in primary (Gerrard et al., 2017) and secondary care (Grossman et al., 2020). In both cases, authors collected data for benchmarking purposes, generally garnering positive feedback from patients

undergoing dental extractions at their respective institutions. While these core

PROMs may play a role in crude service evaluation, they have no proven validity

and reliability and are therefore of limited value in the broader context of patient

outcomes in oral surgery. There is currently no agreed consensus on a core set of

oral surgery PROMs, yet a need to expand the available repertoire of specialty and

procedure-specific PROMs has been identified by the profession (Grossman et al.,

2020).

Question	Response	Details
Did you need to seek advice or assistance hours/days after the procedure?	Yes / No / Unsure	Uncontrolled bleeding (%) Inadequate pain relief that needed further medication (e.g. dry socket? Typically 5% of cases) Infection that needed further treatment (%) Damage to other teeth/fillings (%) Trigeminal erve injury/altered sensation (Typically 1% of cases) TMD
Have you had to have additional surgery subsequent to this treatment?	Yes / No / Unsure	If yes, what is the problem? Fractured jaw Unintentional root retention Bone infection Nerve injury (1%)
Time taken to achieve restoration of normal activities or appearance	Yes / No / Unsure	Days Weeks Months

Table 8. Core Oral Surgery PROMs (NHS England Chief Dental Officer Team, 2015)

PROM development is far from straightforward, and requires a robust analysis of

psychometric properties in the relevant patient population (Ní Ríordáin and

Wiriyakijja, 2017, Higginson and Carr, 2001):

- Validity: does the instrument measure what it is intended to measure?

- Reliability: does the measure produce similar results when repeated in the same population?

- Responsiveness (sensitivity): does the measure detect clinically meaningful changes over time?

- Acceptability: is the measure suitable for its intended purpose?

- Interpretability: are the results measurable and clinically relevant?

This can be achieved by using checklists such as SAC-MOT (Aaronson et al., 2002) or the COnensus-based Standards for the selection of health Measurement INstruments (COSMIN). The latter defines nine measurement properties clustered within three domains (Fig 30). The evaluation process is essential to ensure available PROMs are robust and fit for purpose.

Figure 30. PROM measurement properties defined in COSMIN taxonomy.
HR-PRO, health-related patient reported outcome (Mokkink et al., 2016)

2.4.1 Pain

Crucial to effective postoperative pain management is measurement of a patient's pain intensity. Suitable instruments that equate subjective patient feedback with objective data are essential for this purpose. Patient factors such as ethnicity, level of education and pre-existing affective disorders may influence pain measurement in certain groups (Flaherty, 1996). Perhaps even more importantly for oral surgeons, procedural anxiety has been shown to intensify the postoperative pain experience, and postoperative pain remains the most common reason why patients seek help in the postoperative period (Coulthard et al., 2014b).

Pain has a protective function in the postoperative period by promoting undisturbed healing. For patients undergoing third molar surgery, the pain experience will restrict mouth opening thereby limiting function at the site of surgery and encouraging rest. Reduction of pain after oral surgery, rather than total elimination, is desirable to prevent a premature return to normal function, which might otherwise lead to wound damage and ultimately delayed healing (Coulthard et al., 2014b).

There are two descriptive classes of pain: inflammatory and neuropathic. The former is associated with peripheral tissue damage such as that resulting from oral surgery, and neuropathic pain arises as a result of nervous system dysfunction, such as post-herpetic neuralgia (Coulthard et al., 2014b). The detection and signalling of noxious stimuli (nociception) involves the activation of specialised sensory transducers (nociceptors) that are attached to myelinated (Aδ) and

unmyelinated (C) nerve fibres. Surgically injured tissues release chemicals including bradykinin, serotonin, histamine, prostaglandins, leukotrienes and substance P, which initiate the inflammatory pain process by activating and sensitising nociceptors (Coulthard et al., 2014b).

The following considerations should be taken into account when selecting the most appropriate pain measurement instrument (Flaherty, 1996):

- The dimension of pain being measured e.g. intensity of postoperative inflammatory pain experience (unidimensional); aspects of postcaesarean section pain (multidimensional)
- Type of pain being studied: acute or chronic, inflammatory or neuropathic
- Characteristics of the patient population

2.4.1.1 NRS

The numerical rating scale (NRS) instrument was first described by Downie in 1978, and consists of a linear 11-point scale with either a vertical or horizontal orientation (Fig 31). Patients are asked to rate their pain anywhere from 0 'no pain' to 10 'worst pain imaginable'. The NRS has many practical advantages over its counterparts: it is simple to administer, easy to score and is suitable for written or verbal administration, which is particularly useful for virtual patient appointments. It is also suitable for use by patients whose native language is not English, overcoming any potential language barriers to pain measurement (Flaherty, 1996).
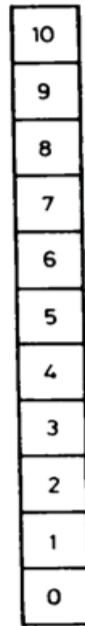
Figure 31. Vertical format of the numerical rating scale (NRS) (Downie et al., 1978)

Its original application was in the assessment of pain in a cohort of 100 rheumatoid arthritis patients, where it was found to outperform the visual analogue scale (VAS) and ordinal descriptor scales (Downie et al., 1978). Other studies have corroborated the superior responsiveness of the NRS compared to other unidimensional pain measuring instruments, and it is recommended as one of the best choices of instrument where sensitive measures of pain intensity are indicated (Ferreira-Valente et al., 2011). The NRS has also demonstrated superior construct validity to the VAS in patients with oral lichen planus (Ní Ríordáin and Wiriyakijja, 2017). In spite of this, an electronic database search failed to generate any studies of autologous platelet concentrate use in third molar surgery where authors selected the NRS as the pain measurement instrument of choice, although NRS use is reported in numerous other non-APC-based third molar studies (Cheung et al., 2011, Lieblich and Danesi, 2017).

2.4.1.2 VAS

The VAS is a 100mm linear scale that has been in use for over 80 years, and is administered in a horizontal or vertical format (Fig 32). It has verbal anchors at either end, "no pain" and "worst pain imaginable", which represent a continuum of pain intensity (Flaherty, 1996). Patients are asked to draw a single mark on the line to indicate their current level of pain. The distance from the "no pain" anchor to this mark is measured using a ruler, which corresponds to the pain score (ranging from 0 to 100). Although the horizontal VAS orientation is the preferred instrument format, its vertical counterpart tends to yield superior sensitivity (Flaherty, 1996).

$$\vdash\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!\dashv$$

No pain   Worst pain imaginable
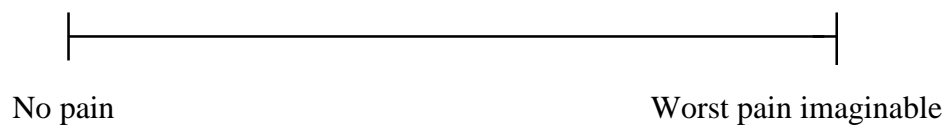
Figure 32. Visual analogue scale

VAS is by far the most commonly cited pain measurement instrument used in third molar surgery studies (Bello et al., 2011, Grossi et al., 2007, Ozgul et al., 2015, Gülşen and Şentürk, 2017, Bilginaylar and Uyanik, 2016, Ritto et al., 2019, Jeyaraj and Chakranarayan, 2018, Dar et al., 2018, Ogundipe et al., 2011). In many instances, researchers give only vague information about the use of VAS in their methods, and the accuracy of VAS scores in such studies is called into question as a result. Jeyaraj and Chakranarayan (2018) in their previously cited study superimpose a faces pain scale onto their VAS, which refutes the validity of the instrument, and is misleading for the readership.

Despite the perceived ubiquity with which the VAS is selected for use in clinical research, it has several disadvantages. Firstly, instructions on how to use the instrument are mandatory to ensure patients understand how to correctly rate their pain level. Secondly, the potential for error when converting a patient's VAS marking to a numeric score cannot be overlooked (Flaherty, 1996). Thirdly, it must be administered in written format if it is to be applied correctly, which renders it useless if the aim is to document pain measurement scores over the telephone. This becomes particularly problematic for patients with limited mobility, those in full-time employment who will miss time from work to attend the clinic, and for patients who live a considerable distance away from the clinic and will incur financial losses as a result.

2.4.1.3 VRS/VDS

The verbal response scale (referred to interchangeably as verbal descriptor scale) was devised by Keele over 70 years ago to measure patient responses to analgesics. It has proven validity and reliability and has the advantage of being simple to use, for both patient and clinician. The scale comprises three to five descriptive words to relay the level of pain intensity such as "none", "mild", "moderate", "severe" and "unbearable" (Flaherty, 1996). The forced responses generated by this instrument are of limited value in circumstances where true elucidation of a patient's pain experience is required.

2.4.1.4 McGill Pain Questionnaire

The McGill Pain Questionnaire (MPQ) has been in use for almost 50 years, and is one of the few instruments that can truly capture the multidimensionality of pain (Flaherty, 1996). It has a long established record of proven validity and reliability across a wide variety of patient groups. The MPQ covers many domains of the pain experience including site and intensity, patterns over time, as well as sensory, affective and miscellaneous aspects of pain. The complexity of the original MPQ and the time required to complete it, prompted the subsequent introduction of an abridged short-form MPQ. The latter documents pain intensity as well as the sensory and affective dimensions of pain by using verbal descriptors such as "throbbing", "shooting", "stabbing", "sharp" and "cramping" (Flaherty, 1996).

The MPQ and its short form equivalent are particularly suited to the evaluation of chronic pain conditions as is seen in patients with malignant disease. No studies investigating the use of APCs in third molar surgery using the MPQ as a pain measurement tool could be found during an electronic literature search. This is probably due to the fact that the more transient, inflammatory pain associated with third molar removal can be suitably captured using a simpler unidimensional instrument such as NRS or VAS (Sirintawat et al., 2017).

2.4.2 Quality of life

Collection of quality of life (QoL) data in third molar surgery has grown in popularity in recent years, thanks in no small part to preliminary reports on the

subject by Savin et al (1997) just over twenty years ago. They found that one week postoperatively, 1 in 7 patients had lost self-confidence, 1 in 3 were not fit to socialise and 1 in 2 required up to one week off work. It is reasonable to assert that the patient is the best assessor of QoL. What a clinician might determine as significant to life quality in the postoperative period differs hugely from that of a patient; indeed this disparity was reported in a survey of 121 dentists and maxillofacial surgeons in Scotland and 120 patients who had undergone third molar surgery. Where clinicians deemed pain to exert the most significant influence on life quality, patients ranked food enjoyment and the ability to eat as the most significant factors (Ogden et al., 1998).

Quality of life assessment encompasses a triad of physical, social and psychological parameters that must be documented preoperatively and postoperatively to be able to truly appreciate the impact of third molar surgery on patients' QoL. A lack of baseline data is identified as a weakness of many QoL studies (Duarte-Rodrigues et al); it is impossible to distinguish a positive impact from a negative one if baseline QoL data are not collected.

Evaluation of QoL in the third molar surgery population has transcended its original brief of application in research studies such as RCTs and cohort studies; it has helped shape what and how we communicate with patients considering third molar surgery. It should be borne in mind that 'cure' is often worse than 'disease' in the case of third molar surgery, and it is imperative that patients are appropriately and adequately informed during the decision-making process (McGrath et al., 2003a).

A systematic review published in 2010 summarised the most commonly used validated QoL PROMs in oral and maxillofacial surgery according to subspecialty interest (Kanatas et al, 2010). Their aim was to create a database of PROMs suitable for use in audit or research, to which clinicians could easily refer. The review reported the following QoL PROMs as those most frequently used in third molar surgery: OHIP-14, OHQoL-UK©, SF-12, MAU and PoSSe.

There is a plethora of QoL instruments currently available for use in third molar surgery that can be broadly categorised into three distinct groups:

- Generic (e.g. SF-12, EQ-5D-3L)

- Oral health-related (e.g. OHIP-14, OHQoL-UK©)

- Disease-specific (e.g. PoSSe)

Disease-specific PROMs have the advantage of demonstrating greater face validity and credibility, while generic PROMs allow for comparisons across conditions (Black, 2013). Oral health-related PROMs meanwhile compare oral operations with other oral healthcare on quality of life (McGrath et al., 2003c). A summary of all PROMs most commonly applied in an oral surgery context is presented in Table 9.

Despite accumulating research on PROMs use in oral surgery, there remains an absence of QoL research on PRGF use in third molar surgery.

Oral symptom-specific

VAS

NRS

QoL
Generic

EQ-5D-3L

SF-36

SF-12

QoL
Oral health-related

OHIP-14

OHQol-UK®

QoL
Disease-specific

PoSSe

Psychosocial-specific

HADS
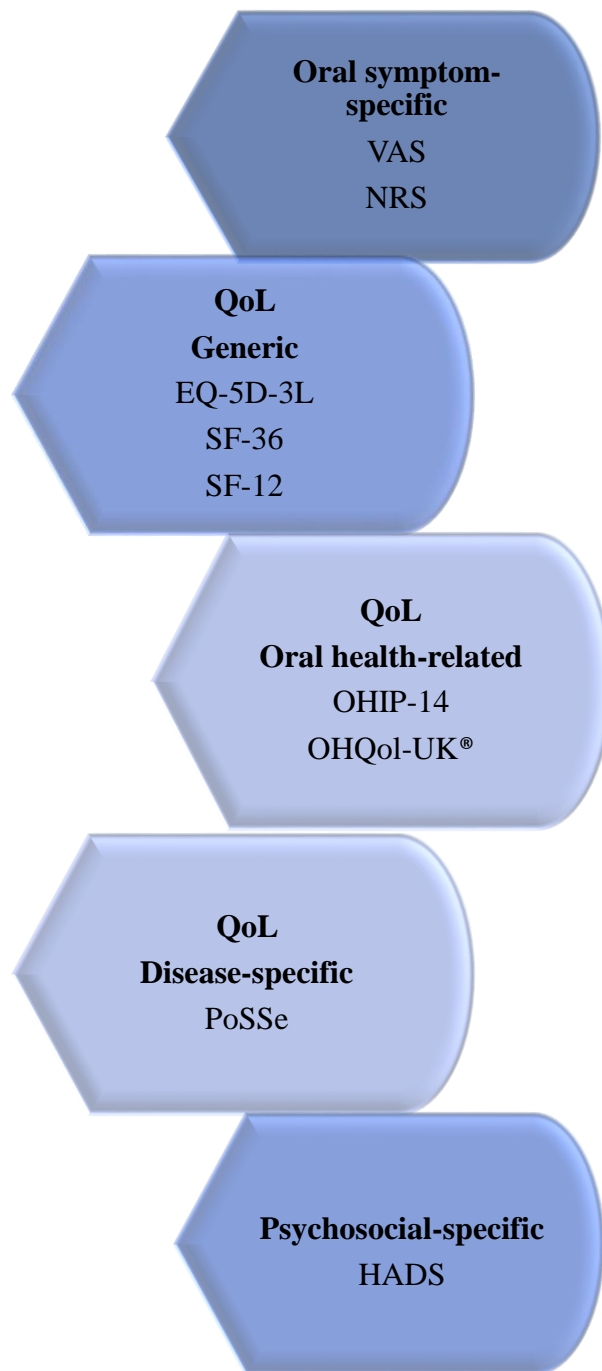
Table 9. Summary of PROMs most commonly reported in oral surgery literature

2.4.2.1 Generic

The EuroQol-5D-3L instrument was introduced in 1990 and is one of the most widely used instruments for measurement of health-related quality of life (EuroQol, 2018). It comprises a descriptive system of five dimensions (Fig 33) and a vertical VAS denoting a patient's self-rated health score at a particular point in time. Each of the five dimensions is scored on a three-point scale, representing 'no problems', 'some problems' and 'extreme problems'. Subsequently EQ-5D-5L, with a novel five response level, was developed to address the potential for ceiling effects and concerns about the sensitivity of the 3L version for detecting clinically important differences in health-related quality of life (Herdman et al., 2011). Recent evidence suggests the 5L version has better measurement properties including feasibility, ceiling effects, sensitivity and convergent validity (Agborsangaya et al., 2014). The use of EQ-5D has increased significantly in recent years as it is not only a means of evaluating quality of life but also a generic preference-based measure (GPBM) used in economic analyses (Yang et al., 2019).

Figure 33. EQ-5D dimensions

Use of the EQ-5D-3L in oral surgery lags far behind that of other healthcare specialties. A study published in 2017 sought to evaluate QoL outcomes in 50 consecutive patients undergoing third molar surgery under general anaesthesia (Beech et al., 2017). With a response rate of 72% (36/50), the authors reported good responsiveness of the EQ-5D-3L instrument. QoL outcomes were worst on the first postoperative day, with a gradual daily improvement observed thereafter during the immediate postoperative week. The 'pain/discomfort' domain demonstrated the greatest impact on postoperative QoL in their cohort. While the authors laud the results of their study, there are some discrepancies in the study design that warrant cautious appraisal of their results: firstly, there are no preoperative QoL data with which to compare outcomes. The true impact of M3M surgery on QoL cannot be fully appreciated without baseline data. Secondly, parallel use of a disease-specific, or oral health-specific instrument would have yielded more meaningful results for the third molar patient population. The EQ-5D-3L instrument for instance does not collect data relating to eating and chewing ability, which has been ranked as one of the most important postoperative outcomes by patients (Ogden, 2014). Thirdly, there are no objective outcome comparators to substantiate their findings, which is another weakness of their study design.

The 36-item short form health survey (SF-36) has been established as a general health measurement instrument for almost thirty years, with a long track record of validity and reliability. It is based on eight health concepts (Brazier et al., 1992):

1. Physical functioning
2. Role limitations due to physical health problems

3. Bodily pain

4. Social functioning

5. General mental health

6. Role limitations due to emotional problems

7. Vitality (energy/fatigue)

8. General health perceptions

Concerns about the feasibility of using the SF-36 in large-scale studies led to subsequent efforts to abridge its content (Ware et al., 1996). Particular focus was given to the physical and mental health components of the instrument, which were reported to account for 80-85% of reliable variance in the eight dimensions in tested populations. The authors reduced the questionnaire length by two thirds with minimal loss in measurement precision, which resulted in a shorter 12-item alternative (SF-12). Selection of SF-12 over its parent SF-36 is advocated in studies with large sample sizes, and in studies evaluating patient-based assessments of physical and mental health status.

The validity and sensitivity of the SF-12 in the context of third molar surgery have previously been investigated (McGrath et al., 2003c). In their cohort of 100 patients awaiting removal of a single M3M under local anaesthesia, the authors found the generic SF-12 instrument was unable to distinguish high-need patients from those who were asymptomatic. They concluded that the SF-12 is not a valid measure of preoperative or postoperative health status in an oral surgery population. It did, however, demonstrate acceptable sensitivity in the immediate postoperative period, correlating well with oral health-related instruments.

2.4.2.2 Oral health-related

The Oral Health Impact Profile (OHIP) is by far the most widely used oral health measure in use today. It was originally developed as a 49-item (OHIP-49) instrument capturing seven conceptually formulated dimensions based on Locker's theoretical model of oral health (Locker, 1988):

1. functional limitation

2. physical pain

3. psychological discomfort

4. physical disability

5. psychological disability

6. social disability

7. handicap

Each of the 49 OHIP items was derived from 535 statements obtained during qualitative interviews with 60 patients (Slade and Spencer, 1994). In numerous epidemiological studies, it has demonstrated tooth loss, barriers to accessing dental care, untreated dental caries and periodontal disease as having the biggest impact on patients' wellbeing.

OHIP-49 has since been largely superseded by the short-form OHIP-14 designed to improve usability without compromising on psychometric properties. OHIP-14 incorporates statements from each of the seven dimensions, which have been shown to be as effective as the OHIP-49 items in detecting differences among subgroups of South Australians aged 60 years and over (Slade, 1997). OHIP-14

demonstrates excellent internal reliability (Cronbach's α = 0.88) and very good validity, reliability and precision and has the added benefit of being more acceptable for use in clinical trials with less respondent burden. Each of the 14 OHIP items is scored from 0 'never' to a maximum of 4 'very often' with higher numbers reflecting a poor quality of life.

OHIP-14 shows superior discriminative validity compared to SF-12 in determining which patients will benefit most from third molar surgery (McGrath et al., 2003c). Preoperative use of oral health measures such as OHIP-14 has been advocated as a screening mechanism to help identify patients most in need of third molar surgery (McGrath et al., 2003c).

The impact of third molar surgery on quality of life has been widely reported in the literature. Authors of a 2018 systematic review on this topic included 13 studies in their evaluation, all of which used OHIP-14 as a validated QoL instrument. A lack of baseline QoL data accounted for a large number of study exclusions (Duarte-Rodrigues et al., 2018). The first reports of oral health-related QoL were documented by McGrath et al in 2003. They collected preoperative and daily postoperative QoL data for 7 days, for 100 consecutive patients awaiting removal of a single impacted lower third molar under local anaesthesia at a single institution, using OHIP-14 and OHQoLUK© instruments. The latter is based on an updated WHO model of 'structure-function-ability-participation', and measures both positive and negative aspects of oral health across 16 domains of life quality (McGrath et al., 2003a). The findings showed the greatest deterioration in QoL on the first postoperative day, which improved gradually but remained

statistically significant for five days (Fig 34). Mean OHIP and OHQoLUK©
scores returned to baseline levels by days 6 and 7 postoperatively. The observed
deterioration in life quality correlated with the clinical outcomes of swelling and
trismus.
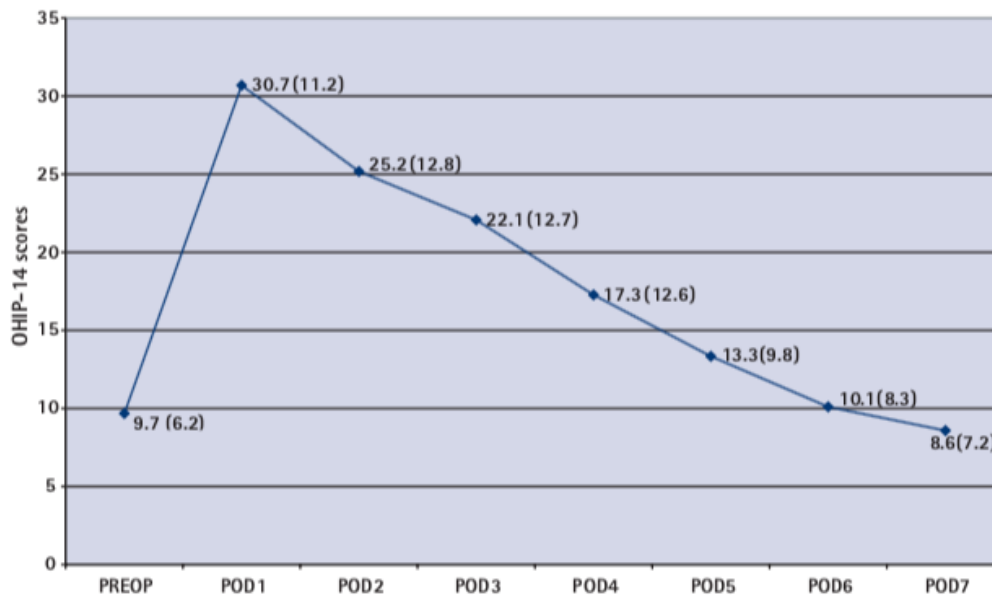
Figure 34. Mean OHIP-14 scores (95% confidence intervals) during the
immediate postoperative week. POD, postoperative day (McGrath et al., 2003a)

The corroboration of these findings in the systematic review by Duarte-Rodrigues
et al (2018) led to their recommendation to patients to withdraw from work and
academic commitments for four days after third molar surgery. There is
conflicting advice surrounding this issue, not least due to the fact that time needed
off work will not only demonstrate huge inter-individual variation, but also depend
on the day of the week on which surgery was performed. An earlier study
investigating ability to work following removal of a single impacted M3M under
local anaesthesia reported the mean duration of inability to work as 1.07 days in a
cohort of 228 patients aged 17-47 years (Berge, 1997). Blanket advice concerning
time from work should therefore be avoided where possible, and instead a common
sense approach adopted where the patient makes his/her own judgement.

OHQoL-UK© was developed twenty years ago by conducting open-ended interviews with 1,865 residents of the United Kingdom (McGrath and Bedi, 2001). A battery of 16 questions was produced, which encompasses four levels: bodily function level (eating, appearance, speech, health, smile), symptom level (comfort, breath), personal level (confidence, personality, mood, carefree, relax/sleep) and social level (social, romance, work, finance). These levels measure 'effect' and 'impact' of oral health on life quality. The instrument demonstrates satisfactory internal reliability (Cronbach's α = 0.94), construct and criterion validity, supporting its implementation in oral health-related quality of life research (McGrath and Bedi, 2001).

Use of OHQoL-UK© in third molar research lags behind that of OHIP-14, and it tends to be favoured as complementary to OHIP-14 rather than preferential in third molar studies (McGrath et al., 2003a, Deepti et al., 2009). OHQoL-UK© measures both positive and negative effects of oral health, whereas OHIP-14 measures only negative attributes (McGrath et al., 2003c). Similarly to the findings by McGrath et al (2003), mean OHQoL-UK© and OHIP-14 scores peaked at day 5 postoperatively in Deepti et al's (2009) cohort of 72 patients undergoing removal of a unilateral impacted M3M, and approximated preoperative values by days 6 and 7 post-surgery.

In a similar manner to that in which dental anxiety is shown to exacerbate patients' pain experience following third molar surgery (Section 2.4.1), higher levels of dental anxiety exert a negative impact on oral health-related quality of life. In a random cohort of 1800 British patients aged 16 years and older, 1 in 10

experienced high levels of dental anxiety, which was associated with lower (worse) OHQoL-UK© scores (McGrath and Bedi, 2004).

2.4.2.3 Disease-specific

There currently exists only one QoL instrument specific to third molar surgery with proven validity, reliability and sensitivity – the Postoperative Symptom Severity (PoSSe) Scale (Ruta et al., 2000). While Ruta et al (2000) acknowledge earlier work by another source in designing an instrument to specifically measure patients' perceptions after third molar removal (Shugars et al., 1996), limitations in its sample size (n=19) and an absence of psychometric testing fail to qualify the instrument as a valid measure of oral health-related QoL.

In devising the PoSSe questionnaire, Ruta et al (2000) followed five steps according to a pre-agreed protocol (Streiner and Norman, 2003):

- Devising the questions
- Testing the questions
- Assessment of internal consistency
- Finalising the questionnaire for clinical use
- Psychometric evaluation

The questions were derived from previous work undertaken by the authors (Ogden et al., 1998), leading to seven subscales whose content validity was confirmed by 120 patients who had undergone bilateral third molar surgery within the previous twelve months. These seven subscales are as follows:

1. Eating

2. Speech

3. Sensation

4. Appearance

5. Pain

6. Sickness

7. Interference with daily activities

The PoSSe achieved a high degree of internal consistency (Cronbach's α = 0.86) among the study cohort of 97 patients aged 18-61 years (Ruta et al., 2000). It demonstrated moderate correlation with four dimensions of the SF-36 (social functioning, physical problems, energy/fatigue and pain) and was more responsive than the latter to clinical change over time. Mean PoSSe scores were significantly related to the number of impacted teeth removed, intraoperative bone removal, presence of bruising and swelling, choice of anaesthesia and consumption of antibiotics. The authors propose the 3-item subscale 'interference with daily activities' may itself serve as a valid yet concise measure of the impact of third molar removal on a patient's general health. They also highlight its merit as a simple audit tool with which to investigate short-term surgical outcomes in clinical practice (Ruta et al., 2000).

In one longitudinal prospective cohort study of over 200 patients undergoing removal of a unilateral impacted M3M, authors were able to confirm the validity and responsiveness of the PoSSe questionnaire (Grossi et al., 2007). Their cohort demonstrated a positive correlation between PoSSe scores and postoperative

trismus and pain experience. The PoSSe has also been used successfully in interventional RCTs; in one study comparing QoL outcomes in 315 patients receiving three different routes of dexamethasone administration (oral, intravenous, submucosal) during third molar removal, authors reported statistical significance in the postoperative PoSSe scores in favour of intravenous and submucosal administration (Brucoli et al., 2019).

The superior responsiveness of the PoSSe instrument over the long-established SF-36 in the context of third molar surgery, together with its rigorous development protocol and psychometric testing, lend favour to its selection as the first choice of instrument for evaluation of the impact of third molar removal on a patient's perceived health (Ruta et al., 2000). While one would expect greater face validity and credibility with a disease-specific PROM (Black, 2013), these qualities are further enhanced by the reproducibility of the PoSSe when used in third molar studies.

2.4.2.4 Psychosocial-specific

The Hospital Anxiety and Depression Scale (HADS) was developed to assess anxiety and depression in patients with illness, and in the general population (Zigmond and Snaith, 1983). It comprises 14 questions divided equally between two subscales: HADS-A (anxiety) and HADS-D (depression). It is recommended in the 2015 NHS Commissioning Guide for use as a routine oral medicine PROM, yet has never been validated for this purpose (Ní Ríordáin and Wiriyakijja, 2017). Nor has it been validated for use in a third molar surgery context. There are reports

of HADS use in oral cancer patients undergoing surgical resection, to fully evaluate depression and anxiety levels during the patient journey. Although anxiety levels tend to stabilise post-surgery, depression levels can increase significantly, in large part due to concerns about facial disfigurement (Kumar et al., 2018). In this context, HADS scores can help determine whether patients are in need of additional psychological support during what can be an incredibly challenging time. It would seem reasonable to extrapolate HADS is best suited to patients with chronic illness, or for those with a protracted surgical journey.

2.4.3 Core outcome sets

This comprehensive review of the third molar literature highlights a need for development of agreed outcome measures for the purposes of clinical research and evidence-based clinical practice. This lack of standardisation of outcomes is not limited to third molar surgery nor indeed to oral surgery but has been acknowledged across healthcare settings leading to the establishment of the Core Outcome Measures in Effectiveness Trials (COMET) Initiative. This is a publicly available registry of agreed outcomes to be used in the provision of healthcare, termed core outcome sets (COS). They are defined as an *"agreed standard set of outcomes that should be measured and reported, as a minimum, in all clinical trials in specific areas of health or healthcare"* (Kirkham et al., 2017). COS play an important role in:

- The standardisation of trial designs, allowing easier reproducibility of results

- Improving comparability across similar trials

- Reducing selective outcome reporting

- Increasing the relevance of results from research which allows pooling of data for systematic reviews and meta-analyses

- Informing healthcare choices

The COS-STAD (Core Outcome Set-STAndards for Development) project has laid out a robust set of recommendations for minimum requirements in the COS development process, irrespective of the area of healthcare to which it may apply. These recommendations cover three domains including scope specification, relevant stakeholders and the consensus process. With commissioners increasingly turning to national CROMs and PROMs data for monitoring of individual hospital performance and case mix, collection of standardised outcome data becomes crucial where hospitals are competing for public funding.

The specialty of oral surgery has a unique opportunity to open discussion with a view to setting standards for researchers. As is the case with other surgical and dental specialties, a steering committee with a panel of experts in the field is essential to drive the necessary steps towards COS development for third molar research and clinical practice. Standardisation of future third molar trials is essential for production of high-quality data that can be merged for meta-analysis, and in turn, inform future clinical practice.

2.5 KNOWLEDGE GAP

Through this comprehensive review of the salient literature, a clear knowledge gap in quality of life outcomes for patients undergoing third molar surgery with adjunctive autologous platelet concentrates, was identified.

2.6 AIMS AND NULL HYPOTHESIS

2.6.1 Aims

1. To determine whether adjunctive use of PRGF in mandibular third molar sockets influences quality of life during the first postoperative week

2. To determine whether adjunctive use of PRGF in mandibular third molar sockets influences postoperative clinical outcomes

The research questions were investigated using the following primary and secondary outcomes:

Primary outcomes:

- NRS pain score

- OHIP-14 score

- PoSSe score

Secondary outcomes:

- Mouth opening

- Dry socket

- Socket healing using the modified Landry et al healing index

- Analgesia consumption

2.6.2 Null hypothesis

Adjunctive use of PRGF in mandibular third molar surgery has no effect on clinical outcomes or patient quality of life during the immediate postoperative period.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN
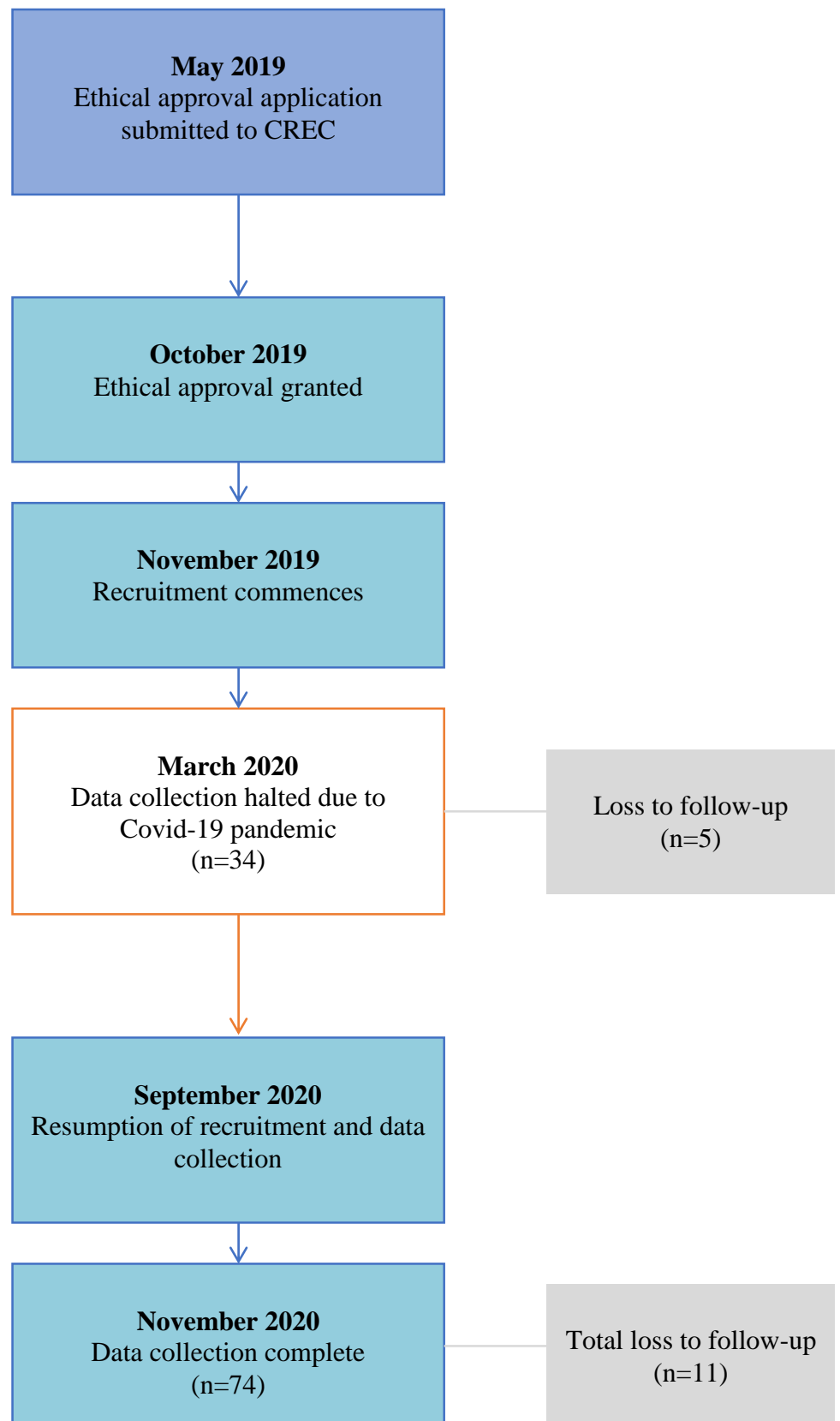
Figure 35 Timeline of research study. CREC, Clinical
Research Ethics Committee

A prospective double-blind randomised controlled clinical trial was designed to investigate whether addition of PRGF to mandibular third molar extraction sockets confers any healing benefit, and in turn to establish what effect this might have on patient quality of life in the immediate postoperative period. A parallel group design was employed in favour of a split-mouth approach, to fully evaluate patient reported outcomes. Ethical approval to undertake this study was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals and data collection commenced in November 2019. Figure 35 provides an overview of the timeline from conception of study design through to completion.

3.1.1 Sample size calculation

The outcome of pain was selected for the purpose of sample size calculation, as it is one of the most common complaints by patients following third molar surgery, with a significant impact on quality of life (O'Sullivan and Ní Ríordáin, 2021). A sample size of 33 patients in each group was calculated to have 80% power to detect a mean difference in the pain numerical rating score (NRS) of 1.5 (SD = 2) using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.05 two-sided significance level. This calculation is consistent with similar previously published third molar research citing pain as the primary outcome measure (Isola et al., 2019). A 10% attrition rate was anticipated, and the target sample size adjusted accordingly.

3.1.2 Recruitment

A convenience sample of 74 eligible patients attending Cork University Dental School and Hospital (CUDSH) were invited to participate in the study during the period November 2019 to November 2020, inclusive. Enrolled participants were recruited from a number of sources including oral surgery consultation clinics, urgent care referrals from the emergency clinic and internal referrals from orthodontic colleagues.

3.1.3 Inclusion criteria

The inclusion criteria were as follows:

- Male or female aged 18-40years

- ASA (American Society of Anaesthesiologists) grade I or II

- Impacted mandibular third molar requiring surgical removal

- No pre-existing temporomandibular joint disorder or other chronic pain condition

- Good command of English language

- Willing to travel for follow-up one week post-operatively

3.1.4 Exclusion criteria

The following criteria deemed patients ineligible for participation in the study:

- Pregnant or breastfeeding women

- Immunosuppression

- Known haematological disorder/coagulopathy

- Current or previous bisphosphonate therapy or history of radiotherapy to the jaws

These criteria were considered in light of all available evidence, and were selected with the intention of generating useful real-life effectiveness data. Many published third molar studies report an upper age limit of 35years (Atalay et al., 2020, Silva de Oliveira et al., 2016, Eshghpour et al., 2018) and 30years (Afat et al., 2019, Armond et al., 2019, Glória et al., 2020, Kaewkumnert et al., 2020) for participants, which we felt would be too restrictive and less generalisable.

3.2 METHOD

3.2.1 T0

All patients enrolled to the study presented to the oral surgery department at CUDSH on the day of treatment (T0) where informed consent for surgical removal of one impacted mandibular third molar was obtained. Each patient was asked to complete a preoperative OHIP-14 and PoSSe questionnaire. Baseline NRS score, maximum incisal opening (MIO) measurement and periodontal probing depth (PPD) distal to the adjacent second molar were recorded by a single clinician.

Patient blinding was achieved by obtaining blood from all participants irrespective of study arm allocation. For each patient, blood was collected into four 9mL tubes containing 3.8% sodium citrate and centrifuged at $580g$ for 8 minutes according to the Endoret® protocol (Figs 36a & b). The platelet-rich Fraction 2 (Fig 36c)

was selectively extracted using the plasma transfer device (PTD) and transferred to a glass dish, where calcium chloride activator was added (2 units per mL Fraction 2). The dish was placed immediately in the oven and the Fraction 2 heated at 37°C for 8-10mins until a 'clot' was formed.

All 74 patients underwent surgical removal of a single impacted mandibular third molar by one of two experienced oral surgeons. Local anaesthesia was administered using 2% xylocaine with 1:80,000 adrenaline via inferior alveolar nerve block and long buccal infiltration. Access to the tooth was achieved by raising a buccal envelope-type full-thickness mucoperiosteal flap. Bone removal and/or tooth/root sectioning were performed where indicated, using a bur in a surgical handpiece with copious saline irrigation, and the tooth delivered using elevators. Curettage of the socket was performed to remove debris, and the socket flushed with saline before wound closure using 4-0 vicryl rapide simple interrupted sutures. Details of the procedure were recorded in all cases by the operating surgeon on a data collection sheet: bone removal (yes/no), tooth sectioning (yes/no) and surgical duration (time from the first incision to the final suture), which was measured by a registered dental nurse using a stopwatch device.

All patients received standard postoperative instructions and a seven-day prescription for paracetamol 1g four times daily, ibuprofen 400mg three times daily, codeine phosphate 30-60mg four times daily and a two-week course of kin 0.2% twice daily antiseptic mouthwash. Patients were asked to record the quantity and frequency of analgesia consumption during the immediate postoperative week.

Figure 36 (a-c). (anticlockwise from top) **a.** Endoret® centrifuge loaded with blood bottles prior to spin **b.** Centrifuged blood samples **c.** Fractionation using plasma transfer device (PTD)
(Photographs taken by author 30th October 2020)

3.2.1.1 Randomisation and blinding

Patients were allocated to one of two study arms via computer-generated randomisation. All patients allocated to the experimental (E) group received the PRGF clot in the third molar extraction socket prior to wound closure. Those allocated to the control (C) group underwent surgical third molar removal in line

with the study protocol but did not receive the PRGF clot or any other socket medicament prior to wound closure.

Study arm allocation was made available only to the operating surgeon via concealed allocation. Brown envelopes denoted male participants and white envelopes denoted female study participants. Within each envelope, instructions were written on a postcard as follows:

- 'PRGF – Yes' indicating allocation to the E group
- 'PRGF – No' indicating allocation to the C group

To mitigate any potential for human error, a sticker was also placed on each postcard using a traffic light system where a green sticker indicates 'PRGF – Yes' and a red sticker indicates 'PRGF – No'. After reviewing the instructions within each envelope, the postcard was discarded immediately in the confidential waste bin by the operating surgeon. The study's principal investigator and all study participants were blinded to the study arm allocation.

3.2.2 T1

All patients received a telephone call three days postoperatively (T0+3). Due to limited clinician availability for the purpose of this research, it was not possible to capture T1 data on postoperative day one, which would have been the ideal scenario in light of available published evidence on postoperative pain experience (see Fig 34). With many of our study participants undergoing surgery on Fridays, it was an unfeasible task to capture data during the first postoperative day, which

would have resulted in unacceptable loss of data. Conversely, in support of our method is the recent report that the majority of published third molar RCTs capture postoperative data on days 2 or 3, plus day 7, postoperatively (O'Sullivan and Ní Ríordáin, 2021). Our timepoints reflect the status quo. A single clinician documented all patients' responses to the OHIP-14 and PoSSe questionnaires, and their postoperative analgesia consumption up to that point. Patients were asked to rate their pain numerically using the NRS, which was recorded on the respective data collection sheet. Appropriate analgesia advice was given over the telephone where indicated to optimise pain management.

3.2.3 T2

Patients were asked to return to the clinic for review seven days postoperatively (T0+7). Clinical inspection was carried out by a single clinician and any complications such as dry socket/alveolar osteitis (AO) documented and managed with local measures. Removal of sutures was performed in cases where localised inflammation was observed and tenderness reported. Wound healing was assessed and graded using a seven-point index devised by Landry et al, with '0' and '7' indicating worst and best outcomes, respectively. MIO and PPD distal to the adjacent mandibular second molar were also recorded.

All patients completed a final OHIP-14 and PoSSe questionnaire, and rated their pain numerically using the NRS scale. Analgesia consumption between T1 and T2 and total number of days off work/college were also recorded for each patient.

3.2.4 Outcome measures

3.2.4.1 CROMs

The CROMs selected in this study were based on a comprehensive review of the literature (see Section 2.3); those measures deemed to best reflect postoperative morbidity following third molar surgery and demonstrating satisfactory reproducibility and reliability were selected in this instance.

3.2.4.1.1 Mouth opening

Measurement of mouth opening is a useful marker of postoperative morbidity following mandibular third molar removal. Trismus (mouth opening less than 30mm) imparts significant functional limitations such as difficulty speaking and an inability to maintain a normal diet. Some authors report a positive correlation between trismus and analgesic consumption in the postoperative period following mandibular third molar removal (Grossi et al., 2007), and it therefore comes as little surprise that mouth opening measurement is a useful predictor of response to therapeutic interventions in clinical trials.

In this study, baseline mouth opening was recorded immediately pre-operatively (T0) by a single clinician. Each patient was asked to first swallow, then to open the mouth as wide as is comfortable. The distance between the incisal edge of the upper right and lower right central incisor teeth was measured in millimetres using

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a disposable plastic ruler, and the value documented on the data collection sheet. This technique was repeated at the 7-day review visit (T2) by the same clinician.

3.2.4.1.2 Periodontal probing depth

Periodontitis is one of the most common indications for third molar surgery, with particular prevalence in horizontal impactions (McArdle et al., 2018a). Some authors report bony periodontal defects along the distal surface of adjacent second molars in up to 44% of cases where the M3M has been removed (Kugelberg et al., 1985). Periodontal probing depth (PPD) measurement is widely used in epidemiological studies due to its reproducibility.

A baseline measurement of PPD in patients requiring M3M removal is indicative of disease and oral hygiene status, particularly in cases of mesioangular and horizontal impaction. In this study, PPD was measured in millimetres immediately pre-operatively (T0) by a single clinician using a Williams periodontal probe. The probe was inserted along the distal aspect of the adjacent second molar tooth until blanching of the gingival tissues was observed, and the measurement recorded in the data collection sheet. The technique was repeated at the 7-day review visit (T2) by the same clinician.

3.2.4.1.3 Alveolar osteitis

Alveolar osteitis (AO), commonly referred to as dry socket, is one of the most common complications following dental extraction, with a particular predilection

for mandibular molar sites (Sharif et al., 2014). It is caused by locally increased fibrinolytic activity within the extraction socket, with a complex interplay of anaerobic bacterial activity also contributing to its pathogenesis (Blum, 2002). Section 2.3.6 outlines the criteria set out by Blum (2002) for diagnosis of AO: *"postoperative pain in and around the extraction site, which increases in severity at any time between 1 and 3 days after the extraction accompanied by a partially or totally disintegrated blood clot within the alveolar socket with or without halitosis"*. Clinically, AO is easily identifiable as a denuded, necrotic socket with a grey discoloration of the surrounding mucosa, with breakdown of the healing fibrin clot and an absence of granulation tissue (Fig 37). In this study, a record was kept of any patients who sought intervention from their general dentist during the first postoperative week for management of AO. In all cases, clinical inspection of the surgical site was performed by a single clinician at the 7-day review visit, and where a diagnosis of AO was made, local measures were employed to manage as appropriate.

Figure 37. Dry socket affecting upper left lateral incisor and first premolar extraction sites three days post-operatively
(Photograph taken by author December 2020)

3.2.4.1.4 Landry et al healing index

Evaluation of surgical site healing is particularly relevant in autologous platelet product interventional studies, due to the many reports of accelerated wound healing owing to their use. A systematic review by del Fabbro et al (2015) reported superior epithelialisation and increased thickness of keratinised tissue at extraction sites treated with PRGF, particularly in the first two weeks postoperatively.

A modification of the healing index devised by Landry et al (1988) has been described by Pippi et al (2015) for application in a dental extraction context. Seven clinical parameters are assessed and scored 0 (bad) or 1 (good) with the highest summative score of 7 indicating excellent healing: gingival colour, granulation tissue, degree of epithelialisation, swelling, bleeding, pain and suppuration. This modified soft tissue healing index was used as a basis for surgical site healing evaluation in this study by a single clinician at the 7-day postoperative review visit (T2), and the score documented on the data collection sheet.

3.2.4.2 PROMs

3.2.4.2.1 NRS

The numerical rating scale (NRS) instrument was first described by Downie in 1978, and consists of a linear 11-point scale with a vertical or horizontal orientation (Section 2.4.1.1). The NRS was selected for use in this study over its more widely used counterpart, the VAS (visual analogue scale) due to its many

practical advantages such as ease of scoring, and application in a written or verbal format, the latter being particularly useful for virtual patient appointments. It is recommended as one of the best choices of instrument where sensitive measures of pain intensity are indicated (Ferreira-Valente et al., 2011).

In this study, all patients were asked to verbally rate their pain level anywhere from 0 'no pain' to 10 'worst pain imaginable' at each of the timepoints T0, T1 and T2. Where patients responded with an answer such as '3 or 4', a value of 3.5 was assigned.

3.2.4.2.2 OHIP-14

The Oral Health Impact Profile (OHIP) is by far the most widely used oral health measure in use today. OHIP-14 is a 14-item scale covering seven psychosocial domains: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap. Each of the 14 OHIP items is scored from 0 'never' to a maximum of 4 'very often' with higher numbers reflecting a poor quality of life. OHIP-14 demonstrates excellent internal reliability (Cronbach's $\alpha = 0.88$) and very good validity, reliability and precision and has demonstrable acceptability of use in clinical trials with minimal respondent burden (Slade, 1997). OHIP-14 has also been advocated as a screening mechanism for identification of patients most in need of third molar surgery (McGrath et al., 2003c).

Baseline preoperative OHIP-14 data were collected for all 74 patients in this study (T0). On day 3 postoperatively (T1), each patient gave verbal responses to OHIP-14 questionnaire over the telephone, which were documented by a single clinician. Previous studies have demonstrated a statistically significant deterioration in quality of life for up to five days postoperatively (McGrath et al., 2003b), and it was anticipated that any impact on quality of life would be captured on postoperative day 3 in this study cohort. All patients attending the 7-day postoperative review visit (T2) were again asked to complete the OHIP-14 questionnaire. Collection of QoL data was undertaken at three timepoints to allow observation of any patterns emerging in life quality during the immediate postoperative week.

3.2.4.2.3 PoSSe

The Postoperative Symptom Severity (PoSSe) scale remains the only disease-specific QoL instrument pertinent to third molar surgery with proven validity, reliability and sensitivity (Ruta et al., 2000). Similar to OHIP-14, it covers seven different subscales: eating, speech, sensation, appearance, pain, sickness and interference with daily activities. The PoSSe is a 15-item questionnaire, and questions are a forced choice, requiring the patient to tick one box; the responses to each forced question are assigned a score ranging from 0 to a number that varies with each question. The scores from all 15 questions are summed, with the result representing a percentage, so a patient ticking the most severe option for each subscale would score 100% and a patient ticking the least severe option for each subscale would score 0%.

Although the PoSSe instrument is designed for use one week postoperatively, baseline data were obtained preoperatively in this study to allow comparisons to be drawn between pre- and postoperative QoL status. All patients were asked to complete a PoSSe questionnaire preoperatively (T0). Verbal responses to the questions were recorded three days postoperatively by a single clinician (T1), and all patients attending for 7-day review (T2) were again asked to complete a PoSSe questionnaire.

3.3 DATA COLLECTION

A summary of the data collected at each time point is presented in table 9.

T0	T1	T2
Patient demographics • Age • Gender • ASA grade • Smoking status • Nationality	PROMs • NRS • OHIP-14 • PoSSe	PROMs • NRS • OHIP-14 • PoSSe
Clinical characteristics • Type of impaction • Pederson classification score • Surgical time • Bone removal (Y/N) • Tooth sectioning (Y/N)	Other • Analgesia consumption • Further treatment	CROMs • MIO • PPD • Healing score • AO
CROMs • MIO • PPD		Other • Analgesia consumption • Days off work/college • Further treatment
PROMs • NRS • OHIP-14 • PoSSe		

Table 10. Overview of data collection. ASA, American society of anaesthesiologists; PROMs, patient-reported outcome measures; NRS, numerical rating score; OHIP-14, 14-item oral health impact profile; PoSSe, post-operative symptom severity scale; Y, yes; N, no; MIO, maximum incisal opening; PPD, periodontal probing depth; AO, alveolar osteitis; CROMs, clinician-reported outcome measures

3.4 STATISTICAL ANALYSIS

Data analysis was performed using IBM SPSS® 25.0 software (SPSS, Chicago, Illinois, USA) and Stata® 15.1. The first set of analyses summarised the demographic characteristics of the subjects in the two groups, along with their outcome values at baseline. Due to randomisation of the subjects into the two groups, the two groups should be balanced at this timepoint, and thus no formal statistical comparisons were performed.

Subsequent analyses examined the difference in outcomes between groups at 3-days post-procedure (time 1) and at 7-days post procedure (time 2), and statistical significance set at $p < 0.05$.

The primary outcomes were NRS pain score and measures from the OHIP-14 and PoSSe questionnaires. The total score and subscales from these scales were continuous in nature. To allow for baseline (pre-operative) scores, the analyses were performed using Analysis of Covariance (ANCOVA). The score at the post-procedure timepoint (either day 3 or day 7) was considered as the outcome variable, with the equivalent score at baseline used as a covariate. When the assumptions of this method were met, the analyses were performed with the outcome on the original scale of measurement. However, when the assumptions were not met (for example if the residuals were not normally distributed), this was typically due to the outcomes having positively skewed distributions. In such instances, the analyses were performed on the log scale. Equivalent statistical

methods were used for the secondary outcomes measured on a continuous scale, where there was a measurement at baseline to allow for.

In addition to the subscales of the OHIP-14 score, a series of individual binary questions were also analysed. A similar approach was taken in that the post-procedure value was considered as the outcome, with the baseline value as a covariate. Due to the binary nature of these outcomes, different analysis methods were required. A general linear model (GLM) was used assuming a binomial outcome and a log link function. This was used in order to express the group differences as risk ratios, which might be more interpretable than expressing the differences as odds ratios (which would be obtained from a logistic regression analysis).

A number of the other secondary outcomes were measured post-procedure only, with no baseline value. Continuous outcomes with no baseline value were compared between groups using the unpaired t-test if found to be normally distributed, or the Mann-Whitney test if not found to follow a normal distribution. Categorical outcomes with no baseline value were analysed using the Chi-square test.

CHAPTER FOUR

RESULTS

4.1 OVERVIEW

Systematic exploration of the data generated various subsets of results, which are presented in sequence. The first set of analyses summarises patient demographics, clinical characteristics and surgical data. It is assumed that groups are balanced at the outset due to randomisation, and thus no formal statistical comparisons are made. The next section presents the various patient- and clinician-reported outcomes at the specified study timepoints: baseline (T0), postoperative day 3 (T1) and postoperative day 7 (T2).

4.2 PATIENT DEMOGRAPHICS

4.2.1 Age

A total of 74 patients enrolled in the study with a mean age of 28.1 years (range 19-39). The mean age of patients in the experimental (PRGF) group was lower than that of the control (non-PRGF) group at 26.7 and 29.5 years, respectively. Summary statistics for age among the groups are displayed in Table 11.

Age (years)	Total (n=74)	Control Group (n=38)	PRGF Group (n=36)
Mean	28.1	29.5	26.7
SD	5.8	6.4	4.8
Range	19-39	19-39	20-37

Table 11. Age profile of study population

4.2.2 Gender

Of the 74 patients who participated in the study, 57 (77%) were female and 17 (23%) male. Gender stratification was achieved between the two study arms with 29 (76.3%) females and 9 (23.7%) males in the control group versus 28 (77.8%) females and 8 (22.2%) males in the PRGF group. Gender data are presented in Table 12.

Gender	Total (n=74)		Control Group (n=38)		PRGF Group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
Female	57	77	29	76.3	28	77.8
Male	17	23	9	23.7	8	22.2
Total	74	100	38	100	36	100

Table 12. Gender distribution among study population with stratification for gender between study arms

4.2.3 Ethnicity

The majority of study participants were Irish, accounting for 66 of the total 74 participants (89.2%). The remainder were Malaysian (n=3, 4.05%), British (n=2, 2.7%), Polish (n=1, 1.35%), Somalian (n=1, 1.35%) and Irish-Burmese (n=1, 1.35%). The distribution of nationalities was relatively consistent between control and PRGF groups (Table 13).

Ethnicity	Total (n=74)		Control Group (n=38)		PRGF Group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
Irish	66	89.2	34	89.5	32	88.9
Malaysian	3	4.05	0	0	3	8.3
British	2	2.7	2	5.3	0	0
Polish	1	1.35	1	2.6	0	0
Somalian	1	1.35	0	0	1	2.8
Irish-Burmese	1	1.35	1	2.6	0	0
Total	74	100	38	100	36	100

Table 13. Ethnicity of study participants, with breakdown of nationalities in control and experimental study arms

4.2.4 Smoking status

A total of 23 smokers were identified in the study cohort (31.1%), with a slightly higher proportion of smokers in the PRGF group (n=12, 33.3%) compared to the control group (n=11, 28.9%).

Smoker	Total (n=74)		Control group (n=38)		PRGF group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
Yes	23	31.1	11	28.9	12	33.3
No	51	68.9	27	71.1	24	66.7
Total	74	100	38	100	36	100

Table 14. Summary of smoking status among study population, with breakdown between study arms

4.3 CLINICAL CHARACTERISTICS

4.3.1 ASA status

The study was restricted to patients categorised as ASA I or II only, implying overall good health or mild systemic disease, respectively. Of the 74 study participants, 41 (55.4%) were classified as ASA grade I, and the remaining 33 (44.6%) patients ASA grade II. A slightly higher percentage of ASA I participants

were allocated to the PRGF group (n=21, 58.3%) compared to the control group
(n=20, 52.6%).

ASA grade	Total (n=74)		Control group (n=38)		PRGF group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
I	41	55.4	20	52.6	21	58.3
II	33	44.6	18	47.4	15	41.7
Total	74	100	38	100	36	100

Table 15. ASA status of study participants

4.3.2 Tooth impaction and Pederson scores

In order to determine the level of surgical difficulty, M3M impaction type was
documented for all patients according to Winter's classification (Figure 38).
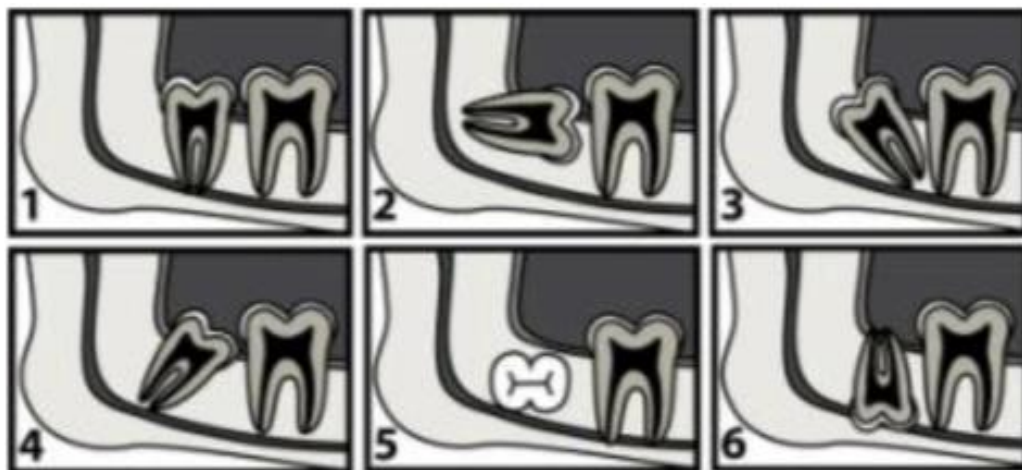
Figure 38. Winter's classification of mandibular third molar impaction. Third
molars are classified according to their inclination relative to the long axis of the
adjacent second molar. 1. Vertical, 2. Horizontal, 3. Distoangular, 4.
Mesioangular, 5. Transverse, 6. Inverse (Miclotte et al., 2017)

Collectively among the study cohort, vertical impaction accounted for the majority
of cases (n=28, 37.8%), followed by mesioangular (n=19, 25.7%), horizontal
(n=15, 20.3%) and distoangular (n=12, 16.2%) impaction. Similar trends are seen
within the control and PRGF groups, but with a slight increase in the number of

horizontal impactions in the latter (n=9, 25%). The findings are summarised in Table 16.

Type of impaction	Total (n=74)		Control group (n=38)		PRGF group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
Vertical	28	37.8	15	39.5	13	36.1
Mesioangular	19	25.7	11	28.9	8	22.2
Horizontal	15	20.3	6	15.8	9	25.0
Distoangular	12	16.2	6	15.8	6	16.7
Total	74	100	38	100	36	100

Table 16. Prevalence of third molar impaction types among study population

The Pederson index was used in this study for classification of surgical difficulty. This index scores third molars based on three criteria: spatial relationship/impaction type, depth of impaction and ramus relationship/space available, generating a score anywhere from 3-10 with higher scores reflecting greater surgical difficulty (Appendix H). Table 17 demonstrates the Pederson scores in this study cohort, with a score of 5 observed most frequently (n=27, 36.5%). Pederson scores remained relatively consistent between the control and PRGF groups.

Pederson score	Total (n=74)		Control group (n=38)		PRGF group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
4	9	12.2	6	15.8	3	8.3
5	27	36.5	13	34.2	14	38.9
6	22	29.7	10	26.3	12	33.3
7	12	16.2	6	15.8	6	16.7
8	4	5.4	3	7.9	1	2.8
Total	74	100	38	100	36	100

Table 17. Pederson scores of study population, with breakdown between study arms

4.4 SURGICAL DATA

All third molar surgeries were performed by one of two experienced oral surgeons (Surgeon 1 and Surgeon 2). Collectively, 34 (46%) surgeries were performed prior to the onset of the global Covid-19 pandemic in March 2020 (see Fig 35). The breakdown of surgeries completed by Surgeon 1 and Surgeon 2 before and after this timepoint is presented in Table 18. Surgeon 1 completed 52 (70%) surgeries in total, 27 before, and 25 during the pandemic. Surgeon 2 completed 22 (30%) of the surgeries overall, 7 before and 15 during the pandemic.

Individual surgeon caseload		SURGEON 1		SURGEON 2		Total
		Control	PRGF	Control	PRGF	
Number of patients	Pre-pandemic	14	13	4	3	34
	During pandemic	12	13	8	7	40
	Total	26	26	12	10	74

Table 18. Breakdown of individual surgeon caseload before and during the global Covid-19 pandemic

4.4.1 Surgical time

The mean surgical time for the entire study population was 13.59 minutes (SD=6.52, range 3.93-30.67) with similar values observed in both control and PRGF groups, at 13.69 minutes and 13.49 minutes, respectively. Table 19 summarises the distribution of surgical times in five-minute time intervals between the study groups. A histogram of collective surgical times is presented in Figure 39.

Surgical time (minutes)	All (n=74)		Control group (n=38)		PRGF group (n=36)	
	Frequency	%	Frequency	%	Frequency	%
<5	4	5.4	3	7.9	1	2.78
5-9.99	23	31.08	13	34.21	10	27.78
10-14.99	18	24.3	5	13.16	13	36.11
15-19.99	15	20.27	8	21.05	7	19.44
20-24.99	11	14.9	7	18.42	4	11.11
25-29.99	2	2.7	1	2.63	1	2.78
30-34.99	1	1.35	1	2.63	0	0
Total	74	100	38	100	36	100

Table 19. Comparison of surgical times between control and study groups
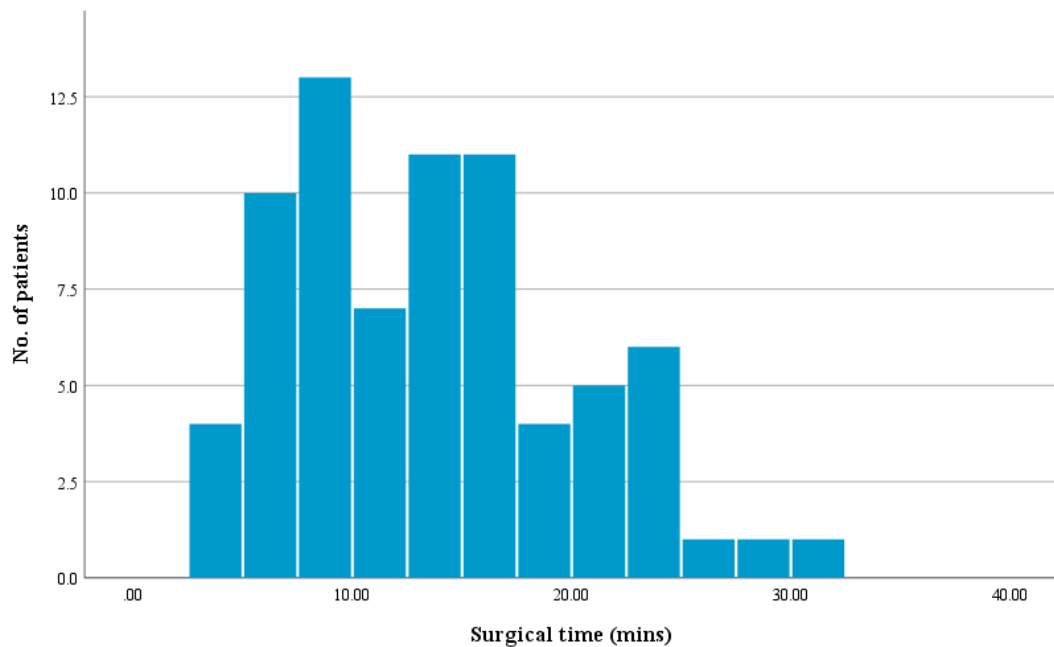
Figure 39. Histogram representation of surgical times for all study participants, demonstrating a slight positive skew. Median surgical time was 12.98mins [IQR 8.0, 17.5]

4.4.2 Surgical technique

All third molar surgeries in this study required lifting of a buccal full-thickness mucoperiosteal flap. The need for bone removal and/or tooth sectioning was determined intraoperatively on a case by case basis, and documented accordingly on the data collection sheet by the operating surgeon in question. Bone removal

was required in 43 (58.1%) cases, and tooth sectioning was performed in 39 (52.7%) cases. Bone removal was required in a slightly greater proportion of the control group (n=23, 60.53%) compared to the PRGF group (n=20, 55.56%). Data related to surgical technique are summarised in Table 20.

Surgical technique	Bone removal						Tooth sectioning					
	All		Control		PRGF		All		Control		PRGF	
	n	%	n	%	n	%	N	%	n	%	n	%
Yes	43	58.1	23	60.53	20	55.56	39	52.7	22	57.89	17	47.22
No	31	41.9	15	39.47	16	44.44	35	47.3	16	42.11	19	52.78
Total	74	100	38	100	36	100	74	100	38	100	36	100

Table 20. Summary of surgical techniques used in both study groups. n, number of patients.

4.5 PRIMARY OUTCOME MEASURES AT BASELINE

4.5.1 NRS pain score

Baseline (T0) NRS values were recorded for all participants to accurately capture trends in pain levels at the postoperative time points. The mean NRS score for the entire study population at baseline was 0.99 (SD=1.61, range 0-6.0); mean NRS score for the control group was 1.22 (SD=1.8, range 0-5.0) and the PRGF group 0.74 (SD=1.37, range 0-6.0). The histogram in Figure 40 demonstrates the skewed distribution of NRS pain scores among the entire study population.
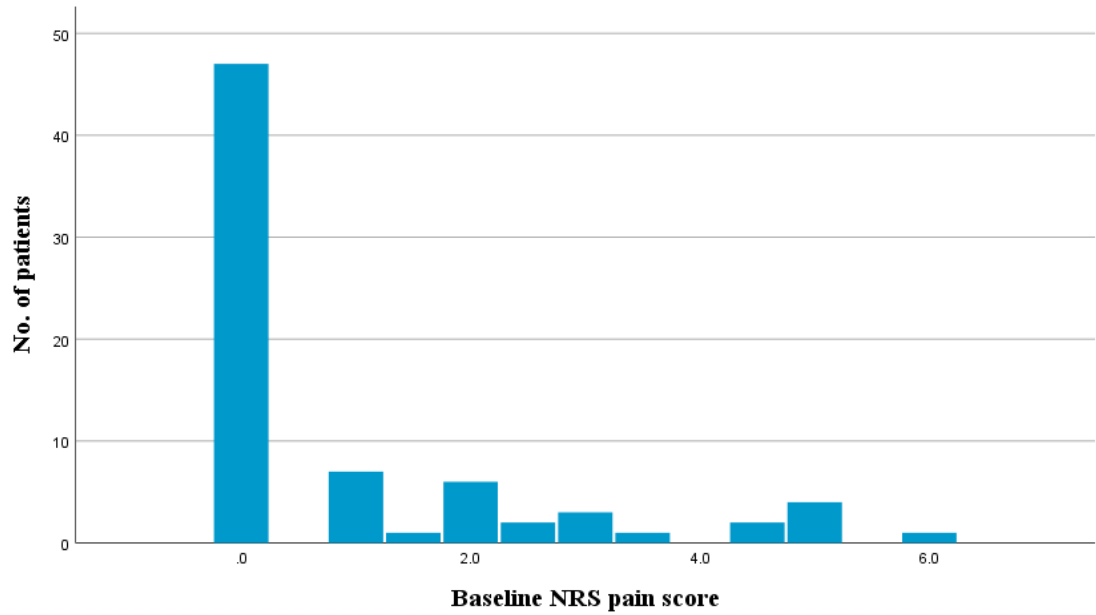
Figure 40. Histogram illustrating the distribution of baseline NRS pain scores among the entire study population, demonstrating a positive skew

The baseline NRS values demonstrated a weak negative correlation with patient age, with higher NRS scores observed in younger participants and vice versa (Fig 41). However, Spearman's correlation coefficient was non-significant (rho=-0.215, p=0.066). Distribution of NRS pain scores was found to be similar between genders (p=0.088). NRS scores at baseline were not influenced by smoking status (p= 0.855).
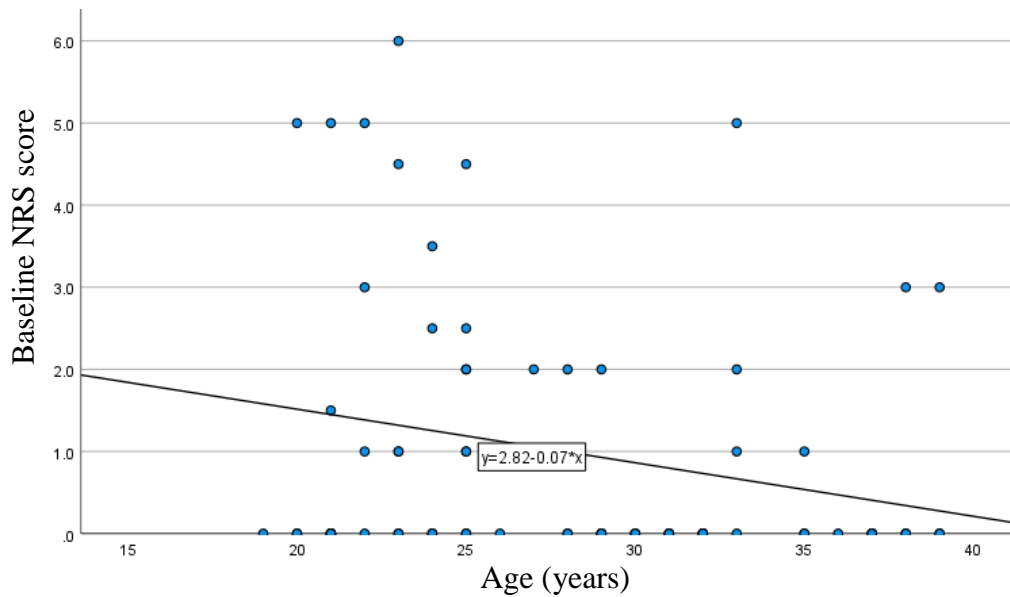
Figure 41. Scatterplot illustrating weak correlation between baseline NRS pain scores and age

4.5.2 OHIP-14 instrument

Responses to the OHIP-14 questionnaire were dichotomised to generate binary values of 0 for the responses 'never' or 'hardly ever' and 1 for 'occasionally, 'fairly often' and 'very often'. Summary statistics for the seven subscales of the OHIP-14 instrument are presented in Table 21, with the 'physical pain' subscale scoring highest in both study groups.

OHIP-14 domains	Control group (n=38)	PRGF group (n=36)
	Median [IQR]	Median [IQR]
Functional limitation	1 [0, 2]	0 [0, 1]
Physical pain	4 [3, 5]	3 [1, 4]
Psychological discomfort	2 [1, 4]	2 [0, 4]
Physical disability	2 [1, 3]	1 [0, 3]
Psychological disability	2 [1, 4]	2 [0, 3]
Social disability	2 [0, 3]	0 [0, 2]
Handicap	1 [0, 2]	0 [0, 2]
Total	15 [8, 21]	9 [4, 16]

Table 21. Summary statistics for OHIP-14 scores at baseline. n, number of patients; IQR, interquartile range

4.5.3 PoSSe instrument

Completed PoSSe questionnaires were later reviewed by a single clinician and corresponding scores assigned to each answer according to the method described by Ruta et al. An excerpt from the PoSSe questionnaire is presented in Fig 42, which shows the values assigned to responses from the 'pain' subscale. For example, a patient experiencing pain for 1-2 days that is completely controlled would score 4.76 (2.38+2.38) in this subscale.

5. PAIN

a.   Thinking of the **last week**, for how many days did you experience **pain** from your operation?
*(Please mark **one** box)*
None at all [0]   1–2 days [2.38]   3–4 days [4.75]   5–6 days [7.13]   7 days [9.5]

b.   Thinking of the **last week**, has the pain from your operation been controlled by **painkillers**?
*(Please mark **one** box)*
I have had no pain. [0]
Yes, completely controlled. [2.38]
Controlled mostly but still some discomfort. [4.75]
Poorly controlled. [7.13]
Not controlled at all. [9.5]

Figure 42. Excerpt from the PoSSe questionnaire demonstrating the weighted scores for 'pain' subscale (Ruta et al., 2000)

Using this method, scores were calculated and recorded for all seven subscales. A combined total score was calculated by adding all the subscale scores together, and expressed as a percentage. Similarly to OHIP-14, 'pain' garnered the highest mean score of all the subscales, followed by 'eating'. Table 22 summarises the baseline PoSSe scores among the study groups.

PoSSe subscales	Control group (n=38)	PRGF group (n=36)
	Mean ± SD	Mean ± SD
Eating	3.3 ± 3.6	3.9 ± 4.7
Speech	0.3 ± 1.1	0.3 ± 1.0
Sensation	0.2 ± 1.3	0.2 ± 0.8
Appearance	0.4 ± 1.1	0.4 ± 1.2
Pain	4.0 ± 5.0	5.1 ± 5.3
Sickness	0.3 ± 1.0	0.3 ± 1.3
Interference with daily activities	1.1 ± 1.8	1.0 ± 1.7
Total	9.6 ± 10.9	11.2 ± 11.8

Table 22. Summary statistics for PoSSe scores at baseline. n, number of patients; SD, standard deviation

4.6 SECONDARY OUTCOME MEASURES AT BASELINE

4.6.1 Mouth opening

Mouth opening (MIO) was recorded for all study participants preoperatively on the day of surgery (T0), and was the principal clinical outcome measure under investigation. The mean baseline MIO among the entire study population was 42.4mm (SD=7.05, range 27-62), with a mean of 41.8mm and 43.1mm for the control and PRGF groups, respectively (Table 23).

Baseline mouth opening (mm)	All (n=74)	Control group (n=38)	PRGF group (n=36)
Mean	42.4	41.8	43.1
Min	27	27	30
Max	62	62	58
Standard deviation	7.05	6.96	7.18

Table 23. Baseline mouth opening measurements of study population

4.6.2 Periodontal probing depth

Similarly, interproximal periodontal probing depth (PPD) between the impacted M3M and the distal surface of the adjacent second molar was measured using a Williams periodontal probe and recorded preoperatively on the day of surgery (T0) for all patients. A mean baseline PPD of 4mm (SD=1.1, range 2-6) was observed in the control group, and 4.3mm (SD=1.6, range 2-8) in the PRGF group (Table 24).

Baseline periodontal probing depth (mm)	All (n=74)	Control group (n=38)	PRGF group (n=36)
Mean	4.16	4.03	4.31
Min	2	2	2
Max	8	6	8
Standard deviation	1.345	1.102	1.564

Table 24. Baseline periodontal probing depths of study population

4.7 PRIMARY OUTCOME MEASURES AT DAY THREE

At postoperative day 3 (T1), all patients received a telephone call from a single clinician where they were asked to rate their current pain level using the NRS scale. OHIP-14 and PoSSe questionnaires were completed verbally. Patients were also asked about their analgesic consumption during the previous 72 hours and number of days taken from work (excluding the day of surgery). All patients in the control group (n=38) were contactable via telephone, while two patients in the PRGF group failed to answer the telephone call (n=34).

4.7.1 NRS pain score

There was slight evidence of a difference in NRS pain scores between groups, although this difference was only of borderline significance (p=0.06). Pain scores were on average 1.0 unit higher in the PRGF group at T1.

Outcome	Treatment	N	Baseline Mean ± SD	3-days post Mean ± SD	Group Difference (*) Mean (95% CI)	P-value
NRS pain	Control	38	1.2 ± 1.8	3.2 ± 2.3	0	0.06
	PRGF	34	0.8 ± 1.4	4.1 ± 2.4	1.0 (-0.1, 2.2)	

Table 25. NRS pain scores at postoperative day three
(*) Calculated from ANCOVA analysis, adjusting for baseline value

4.7.2 OHIP-14 individual questions

OHIP-14 data were collected for 37 patients in the control group and 34 patients in the PRGF group at T1. Table 26 shows the dichotomised outcomes for the 14 individual questions on the OHP-14 questionnaire where the figures are the number of patients with data at T0 and T1. Group differences are expressed as a risk ratio, indicating the likelihood of the outcome occurring in the PRGF group relative to the likelihood in the control group. Corresponding confidence intervals for these relative differences are shown, together with p-values indicating the significance of the results.

Outcome	Treatment	n	Baseline n (%)	3-days post n (%)	Group Difference (*) Risk Ratio (95% CI)	P-value
OHIP-14 Q1	Control	37	4 (11%)	8 (22%)	1	0.31
	PRGF	34	0 (0%)	11 (33%)	1.53 (0.67, 3.45)	
OHIP-14 Q2	Control	37	4 (11%)	8 (22%)	1	0.97
	PRGF	33	6 (18%)	7 (21%)	0.98 (0.40, 2.43)	
OHIP-14 Q3	Control	37	24 (65%)	27 (73%)	1	0.29
	PRGF	34	19 (56%)	27 (79%)	1.14 (0.89, 1.47)	
OHIP-14 Q4	Control	36	22 (61%)	28 (78%)	1	**0.02**
	PRGF	33	15 (45%)	28 (85%)	1.25 (1.04, 1.49)	
OHIP-14 Q5	Control	37	10 (27%)	5 (14%)	1	0.07
	PRGF	34	9 (26%)	11 (32%)	2.39 (0.93, 6.12)	
OHIP-14 Q6	Control	37	11 (30%)	6 (16%)	1	0.53
	PRGF	34	11 (33%)	8 (24%)	1.36 (0.53, 3.47)	
OHIP-14 Q7	Control	37	6 (16%)	15 (41%)	1	0.80
	PRGF	34	5 (15%)	15 (44%)	1.07 (0.63, 1.80)	
OHIP-14 Q8	Control	37	13 (35%)	19 (51%)	1	0.44
	PRGF	34	9 (26%)	17 (50%)	1.16 (0.80, 1.66)	
OHIP-14 Q9	Control	37	14 (38%)	14 (38%)	1	0.83
	PRGF	34	13 (38%)	12 (35%)	0.93 (0.50, 1.73)	
OHIP-14 Q10	Control	37	10 (27%)	3 (8%)	1	0.41
	PRGF	33	10 (30%)	5 (15%)	1.74 (0.46, 6.60)	

OHIP-14 Q11	Control	36	8 (22%)	11 (30%)	1	0.62
	PRGF	34	5 (15%)	9 (26%)	0.84 (0.43, 1.66)	
OHIP-14 Q12	Control	37	10 (27%)	9 (24%)	1	0.08
	PRGF	34	5 (15%)	15 (44%)	1.83 (0.92, 3.63)	
OHIP-14 Q13	Control	37	7 (19%)	11 (30%)	1	0.21
	PRGF	34	7 (21%)	13 (38%)	1.44 (0.81, 2.57)	
OHIP-14 Q14	Control	37	4 (11%)	2 (5%)	1	0.13
	PRGF	34	5 (15%)	6 (18%)	3.27 (0.71, 15.1)	

Table 26. OHIP-14 binary outcomes at postoperative day three
(*) Group difference after adjusting for baseline value

The results suggest that only Question 4 *"have you found it uncomfortable to eat any foods because of problems with your teeth, mouth or dentures?"* was found to significantly vary between groups, with a positive answer more common in the PRGF group. After allowing for the baseline scores, this outcome was 25% more likely in the PRGF group than the control group. There was also slight evidence that Question 5 *"have you been self-conscious because of your teeth, mouth or dentures?"* and Question 12 *"have you had difficulty doing your usual jobs because of problems with your teeth, mouth or dentures?"* were also more common in the PRGF group, but these results did not quite achieve statistical significance.

4.7.3 OHIP-14 subscales

The results of the OHIP-14 subscale analysis are presented in Table 27. Due to the skewed distribution of these outcomes, the data at each timepoint were summarised by the median and interquartile range, and the analysis was performed on the log scale. Due to this transformation, the group differences are expressed as ratios, along with corresponding confidence intervals. These represent the scores in the PRGF group relative to those in the control group. The results did not demonstrate any significant variation in subscale scores or total scores between both groups.

OHIP-14 Outcome	Treatment	n	Baseline Median [IQR]	3-days post Median [IQR]	Group Difference [*] Ratio (95% CI)	P-value
Functional limitation	Control	29	1 [0, 2]	0 [0, 3]	1	0.19
	PRGF	29	0 [0, 0]	1 [0, 3]	1.28 (0.88, 1.86)	
Physical pain	Control	29	4 [3, 4]	5 [3, 6]	1	0.16
	PRGF	29	3 [1, 4]	6 [4, 7]	1.20 (0.93, 1.56)	
Psychological discomfort	Control	29	1 [1, 4]	1 [0, 2]	1	0.44
	PRGF	29	1 [0, 4]	2 [0, 3]	1.16 (0.80, 1.68)	
Physical disability	Control	29	2 [1, 3]	2 [0, 4]	1	0.20
	PRGF	29	1 [0, 2]	2 [0, 6]	1.33 (0.86, 2.06)	
Psychological disability	Control	29	2 [1, 4]	1 [0, 3]	1	0.88
	PRGF	29	2 [0, 2]	1 [0, 3]	1.03 (0.69, 1.53)	
Social disability	Control	29	1 [0, 3]	1 [0, 3]	1	0.20
	PRGF	29	0 [0, 1]	1 [0, 4]	1.30 (0.86, 1.97)	
Handicap	Control	29	1 [0, 2]	0 [0, 2]	1	0.27
	PRGF	29	0 [0, 2]	0 [0, 3]	1.25 (0.84, 1.85)	
Total score	Control	29	15 [8, 20]	16 [5, 20]	1	0.10
	PRGF	29	8 [4, 15]	15 [8, 28]	1.44 (0.93, 2.22)	

Table 27. OHIP-14 subscales at postoperative day three
[*] Calculated from ANCOVA analysis, adjusting for baseline value

4.7.4 PoSSe instrument

PoSSe questionnaires were completed at T1 by a single clinician by obtaining verbal responses over the telephone. Data were collected for 38 patients in the control group and 34 patients in the PRGF group. ANCOVA analysis was performed on each of the seven PoSSe subscales and the total PoSSe score. T1 values were considered as the outcome variable, with the equivalent scores at T0 used as a covariate. The results of this analysis are presented in Table 28, which shows the groups did not vary significantly for the total score or for the majority of the subscales. The exception was the 'interference with daily activities' subscale, where a statistically significant difference was observed. The outcome values for this subscale were significantly higher in the PRGF group, with a mean difference of 1.2 between the groups.

PoSSe Outcome	Treatment	n	Baseline Mean ± SD	3-days post Mean ± SD	Group Difference [*] Mean (95% CI)	P-value
Eating	Control	38	3.3 ± 3.6	9.4 ± 4.4	0	0.10
	PRGF	34	4.0 ± 4.8	11.2 ± 3.8	1.6 (-0.3, 3.4)	
Speech	Control	38	0.3 ± 1.1	1.0 ± 1.2	0	0.12
	PRGF	34	0.3 ± 1.1	1.5 ± 1.4	0.5 (-0.1, 1.1)	
Sensation	Control	38	0.2 ± 1.3	2.0 ± 2.2	0	0.59
	PRGF	34	0.2 ± 0.8	2.3 ± 1.9	0.3 (-0.7, 1.2)	
Appearance	Control	38	0.4 ± 1.1	2.7 ± 1.5	0	0.58
	PRGF	34	0.4 ± 1.2	2.1 ± 1.3	-0.2 (-0.8, 0.5)	
Pain	Control	38	4.0 ± 5.0	7.6 ± 2.7	0	0.89
	PRGF	34	5.0 ± 5.4	7.8 ± 3.2	0.1 (-1.3, 1.5)	
Sickness	Control	38	0.3 ± 1.0	0.9 ± 1.8	0	0.43
	PRGF	34	0.4 ± 1.3	1.3 ± 2.1	0.3 (-0.5, 1.2)	
Interference with daily activities	Control	38	1.1 ± 1.8	2.5 ± 2.1	0	**0.02**
	PRGF	34	1.0 ± 1.7	3.7 ± 2.3	1.2 (0.2, 2.2)	
Total score	Control	38	9.6 ± 10.9	26.2 ± 10.9	0	0.13
	PRGF	34	11.4 ± 12.2	30.3 ± 10.0	3.5 (-1.1, 8.1)	

Table 28. PoSSe subscales and total scores at postoperative day three
(*) Calculated from ANCOVA analysis, adjusting for baseline value

4.8 SECONDARY OUTCOME MEASURE AT DAY THREE

4.8.1 Analgesia consumption

Analgesia consumption was recorded at T1 and T2 only and therefore had no equivalent baseline values. This secondary outcome was analysed using the Mann-Whitney test as data did not follow a normal distribution. Paracetamol was prescribed as a 1g dose, ibuprofen at 400mg and codeine at 30mg. The results of this analysis are summarised in Table 29, which suggested no significant differences in analgesia consumption between the two study groups.

| Outcome | Control group | | PRGF group | | Difference [*] | P- |
	n	Median [IQR]	n	Median [IQR]	Median (95% CI)	Value
Paracetamol	38	13 [6, 20]	34	12 [5, 20]	0 (-4, 4)	0.99
Ibuprofen	38	6 [4, 9]	34	8 [6, 8]	1 (-1, 2)	0.39
Codeine	38	6 [0, 9]	34	6 [2, 10]	0 (-2, 3)	0.58

Table 29. Postoperative analgesia consumption at day three. Analgesic doses were prescribed as follows: paracetamol 1g, ibuprofen 400mg, codeine 30mg
[*] Difference calculated as: value for PRGF minus value for Control

4.9 PRIMARY OUTCOME MEASURES AT DAY SEVEN

4.9.1 NRS pain score

Sixty-three patients returned for postoperative review on postoperative day 7 (T2). Analysis of NRS pain scores at T2 showed no statistical significance between the control and PRGF groups. Mean NRS scores for the control group were identical at T1 and T2 at 3.2 (SD= 2.3, 2.6, respectively). In contrast, there was a reduction in mean NRS score of 1.4units among the PRGF cohort from T1 to T2 with values

of 4.1 (SD=2.4) and 2.7 (SD=2.2), respectively. The results of the T2 analysis are presented in Table 30.

Outcome	Treatment	n	Baseline Mean ± SD	7-days post Mean ± SD	Group Difference [*] Mean (95% CI)	P-value
NRS pain	Control	33	1.3 ± 1.9	3.2 ± 2.6	0	0.44
	PRGF	33	0.8 ± 1.4	2.7 ± 2.2	-0.5 (-1.7, 0.7)	

Table 30. NRS pain scores at postoperative day seven
 (*) Calculated from ANCOVA analysis, adjusting for baseline value

Figure 43 demonstrates individual pain experience among patients in the control group from baseline to the end of the study period. Fifteen patients reported peak pain on postoperative day 3 (T1), 11 patients experienced peak pain on postoperative day 7 (T2), 5 patients experienced peak pain at baseline (T0), 1 patient had equivalent pain score at T1 and T2, and 1 patient experienced no pain at any of the timepoints. NRS scores could not be recorded for 5 patients at T2 due to failure to return for follow-up.
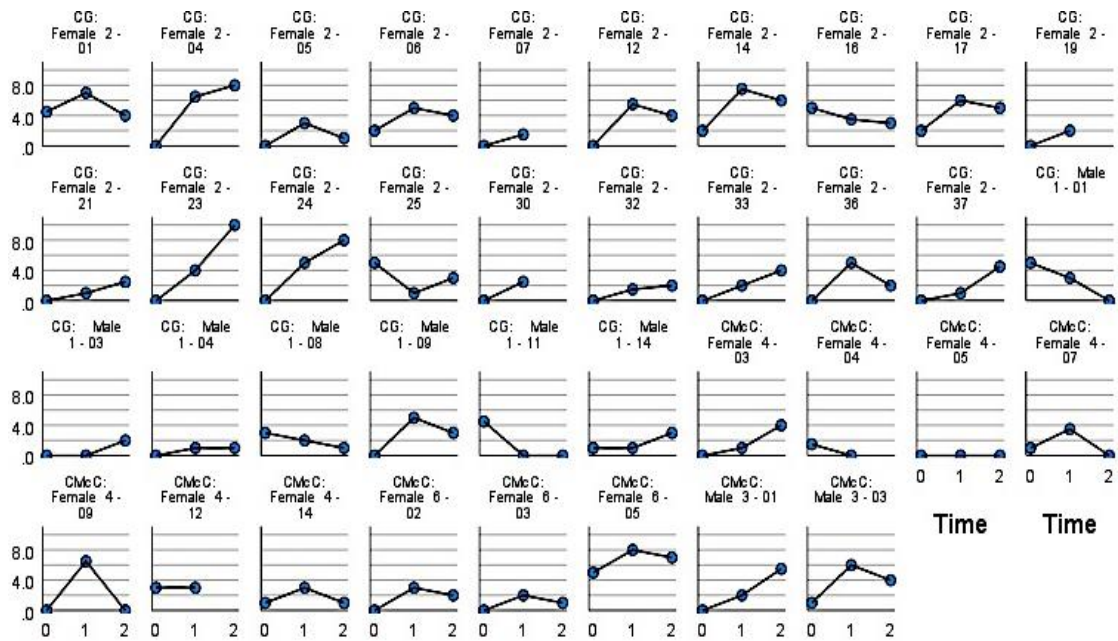
Figure 43. Individual scatterplots showing NRS trends in the control group from baseline to the end of the study period. X-axes correspond to timepoints where 0=baseline, 1=postoperative day 3, 2=postoperative day 7. Y-axes represent NRS scores at each timepoint.

Individual pain experience for the PRGF group is similarly presented in scatterplot format in Figure 44. For 20 patients, peak pain was reported at postoperative day 3 (T1), 6 patients reported peak pain at postoperative day 7 (T2) and 2 patients reported peak pain at baseline. Four further patients reported peak pain at T1 and T2, while one reported peak pain at T0 and T1. The remaining 3 patients failed to return for follow-up at T2.

159
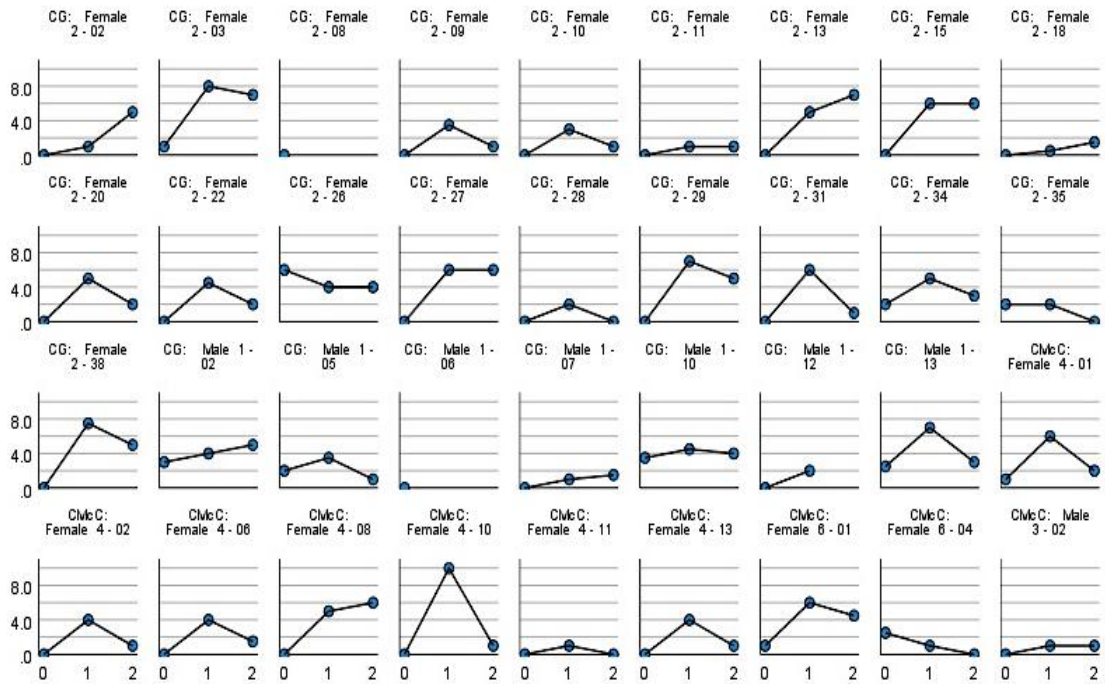
Figure 44. Individual scatterplots showing NRS trends in the PRGF group from baseline to the end of the study period. X-axes correspond to timepoints where 0=baseline, 1=postoperative day 3, 2=postoperative day 7. Y-axes represent NRS scores at each timepoint.

The following graph (Fig 45) shows the trends in estimated marginal mean NRS scores in both the PRGF and control groups. Variations in NRS pain scores are not statistically significant at any timepoint (T1 p=0.06, T2 p=0.44). A marginally sharper peak is observed in the PRGF group at T1 with a considerable drop by T2, while in the control group a more gradual improvement in NRS pain score is observed between T1 and T2.

160
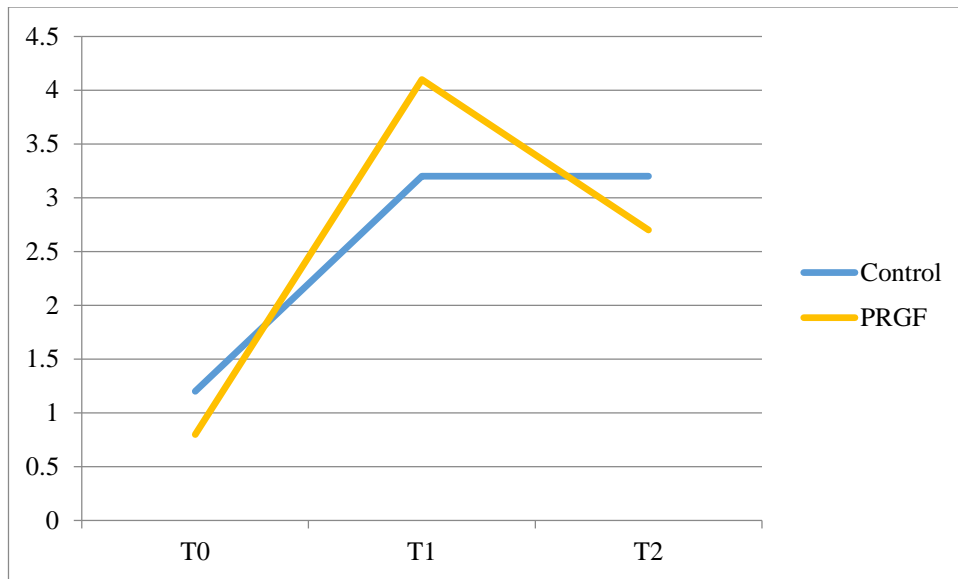
Figure 45. Line chart representation of mean NRS pain scores in PRGF and control groups showing slightly higher peak NRS pain scores in the PRGF group at T1. X-axis, study timepoints; y-axis, mean NRS pain scores

4.9.2 OHIP-14 individual questions

OHIP-14 scores were recorded for 33 patients in each of the study groups on postoperative day 7 (T2), with missing values noted for Q2, Q4 and Q10. The remaining 8 patients did not return for follow-up and were not contactable via telephone. Statistical analysis of the binary components of the OHIP-14 questionnaire at T2 suggested no strong evidence that any of the 14 scores varied between groups. The results from this analysis are presented in Table 31.

Outcome	Treatment	n	Baseline n (%)	7-days post n (%)	Group Difference [*] Risk Ratio (95% CI)	P-value
OHIP-14 Q1	Control PRGF	33 33	4 (12%) 0 (0%)	8 (24%) 7 (21%)	1 1.03 (0.39, 2.70)	0.96
OHIP-14 Q2	Control PRGF	33 32	4 (12%) 6 (19%)	12 (36%) 7 (22%)	1 0.58 (0.28, 1.19)	0.14
OHIP-14 Q3	Control PRGF	33 33	21 (64%) 19 (57%)	27 (82%) 26 (79%)	1 1.15 (0.97, 1.33)	0.07
OHIP-14 Q4	Control PRGF	32 32	21 (66%) 15 (47%)	26 (81%) 24 (75%)	1 0.98 (0.76, 1.26)	0.88
OHIP-14 Q5	Control PRGF	33 33	11 (33%) 9 (27%)	7 (21%) 4 (12%)	1 0.61 (0.20, 1.84)	0.38
OHIP-14 Q6	Control PRGF	33 33	11 (33%) 11 (33%)	11 (33%) 9 (27%)	1 0.78 (0.37, 1.62)	0.50
OHIP-14 Q7	Control PRGF	33 33	5 (15%) 5 (15%)	14 (42%) 17 (52%)	1 1.14 (0.73, 1.80)	0.56
OHIP-14 Q8	Control PRGF	33 33	13 (39%) 9 (27%)	19 (58%) 16 (48%)	1 0.96 (0.62, 1.48)	0.84
OHIP-14 Q9	Control PRGF	33 33	13 (39%) 13 (39%)	17 (52%) 18 (55%)	1 1.12 (0.74, 1.69)	0.61
OHIP-14 Q10	Control	33	10 (30%)	8 (24%)	1	0.46

	PRGF	32	10 (31%)	6 (19%)	0.71 (0.28, 1.76)	
OHIP-14 Q11	Control	33	8 (24%)	13 (39%)	1	0.74
	PRGF	33	5 (15%)	12 (36%)	1.10 (0.64, 1.88)	
OHIP-14 Q12	Control	33	9 (27%)	12 (36%)	1	0.37
	PRGF	33	5 (15%)	15 (45%)	1.30 (0.73, 2.29)	
OHIP-14 Q13	Control	33	6 (18%)	9 (27%)	1	0.58
	PRGF	33	7 (21%)	10 (30%)	1.22 (0.60, 2.49)	
OHIP-14 Q14	Control	33	4 (12%)	5 (15%)	1	0.76
	PRGF	33	5 (15%)	6 (18%)	0.87 (0.35, 2.15)	

Table 31. OHIP-14 binary outcomes at postoperative day seven
(*) Group difference after adjusting for baseline value

4.9.3 OHIP-14 subscales

Analysis of the OHIP-14 subscale scores and total scores was performed next, with

no suggestion of a significant difference between the two groups.

OHIP-14 Outcome	Treatment	n	Baseline Median [IQR]	7-days post Median [IQR]	Group Difference [*] Ratio (95% CI)	P-value
Functional limitations	Control	26	1 [0, 2]	2 [0, 3]	1	0.52
	PRGF	28	0 [0, 0.5]	0.5 [0, 2.5]	0.89 (0.61, 1.28)	
Physical pain	Control	26	4 [3, 5]	5.5 [3, 7]	1	0.85
	PRGF	28	3 [1, 4]	5 [3, 7]	1.03 (0.76, 1.38)	
Psychological discomfort	Control	26	2 [1, 4]	2 [0, 3]	1	0.43
	PRGF	28	1.5 [0, 4]	1 [0, 3]	0.87 (0.60, 1.25)	
Physical disability	Control	26	2 [1, 3]	2.5 [1, 5]	1	0.56
	PRGF	28	1 [0, 2.5]	3.5 [0, 5.5]	1.14 (0.73, 1.78)	
Psychological disability	Control	26	2.5 [1, 4]	2 [1, 4]	1	0.62
	PRGF	28	2 [0, 2.5]	2 [0, 3]	0.91 (0.63, 1.33)	
Social disability	Control	26	2 [0, 3]	2 [1, 5]	1	0.37
	PRGF	28	0 [0, 1.5]	2.5 [0, 4]	1.21 (0.80, 1.82)	
Handicap	Control	26	1 [0, 2]	1 [0, 2]	1	0.50
	PRGF	28	0 [0, 2]	1 [0, 3]	1.15 (0.76, 1.74)	
Total score	Control	26	16 [8, 21]	19.5 [8, 28]	1	0.86
	PRGF	28	8.5 [4.5, 15.5]	16.5 [4.5, 29]	1.05 (0.62, 1.77)	

Table 32. OHIP-14 subscales at postoperative day 7
(*) Calculated from ANCOVA analysis, adjusting for baseline value

Figure 46 offers a graphical representation of the trends in OHIP-14 total scores among both study groups at the three study timepoints. Total OHIP-14 scores were on average higher in the control group at baseline (T0) indicating marginally worse baseline QoL in this cohort. OHIP-14 scores increase in both groups from T0 to T1, and again from T1 to T2.
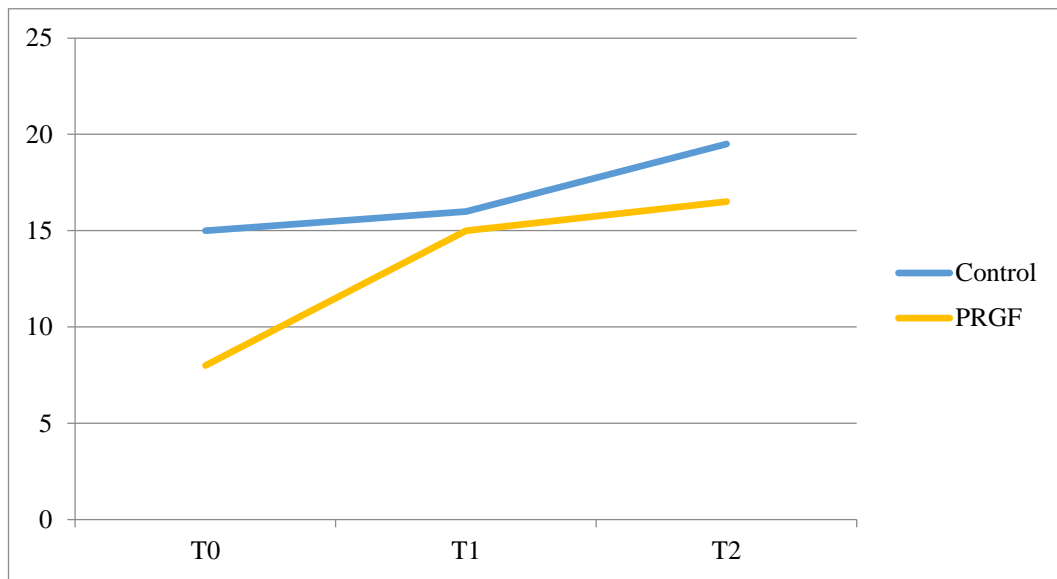
Figure 46. Line chart representing trends in total OHIP-14 scores in both study groups at all three timepoints. X-axis, study timepoints; y-axis, median OHIP-14 total scores

4.9.4 PoSSe instrument

Of the seven PoSSe subscales, 'eating' and 'pain' attracted the highest scores in both study groups one week postoperatively. Mean scores of 11.4 (SD=5.8) and 12.0 (SD= 5.7) for 'eating' (p=0.71) and 10.9 (SD 4.1) and 10.8 (SD 4.1) for 'pain' (p=0.93) were observed in the control and PRGF groups, respectively. PoSSe outcomes (subscales and total scores) did not vary significantly between the control and PRGF groups. A mean total PoSSe score of 33.2 (SD=15.5, range 7.4-61.3) was observed in the control group at T2, and a mean of 35.1 (SD=15.0, range

2.4-61.4) observed in the PRGF group. Table 33 presents the results of ANCOVA

analysis of PoSSe subscales and total scores at T2.

PoSSe Outcome	Treatment	n	Baseline Mean ± SD	7-days post Mean ± SD	Group Difference (*) Mean (95% CI)	P-value
Eating	Control	33	3.3 ± 3.5	11.4 ± 5.8	0	0.71
	PRGF	33	4.1 ± 4.8	12.0 ± 5.7	0.5 (-2.3, 3.4)	
Speech	Control	33	0.3 ± 1.2	1.4 ± 1.9	0	0.37
	PRGF	33	0.3 ± 1.1	1.8 ± 1.9	0.4 (-0.5, 1.3)	
Sensation	Control	33	0.0 ± 0.0	2.1 ± 2.6	0	0.95
	PRGF	33	0.2 ± 0.8	2.0 ± 1.8	0.0 (-1.2, 1.1)	
Appearance	Control	33	0.4 ± 1.2	3.1 ± 2.4	0	0.70
	PRGF	33	0.4 ± 1.2	3.3 ± 2.0	0.2 (-0.9, 1.3)	
Pain	Control	33	4.5 ± 5.2	10.9 ± 4.1	0	0.93
	PRGF	33	5.2 ± 5.5	10.8 ± 4.1	-0.1 (-2.1, 1.9)	
Sickness	Control	33	0.2 ± 0.7	1.2 ± 1.8	0	0.96
	PRGF	33	0.4 ± 1.3	1.3 ± 2.0	0.0 (-0.9, 0.8)	
Interference with daily activities	Control	33	1.1 ± 1.6	3.2 ± 2.5	0	0.26
	PRGF	33	1.0 ± 1.7	3.9 ± 2.9	0.8 (-0.6, 2.1)	
Total score	Control	33	9.8 ± 10.7	33.2 ± 15.5	0	0.64
	PRGF	33	11.7 ± 12.2	35.1 ± 15.0	1.8 (-5.8, 9.4)	

Table 33. PoSSe subscales and total scores at postoperative day seven
(*) Calculated from ANCOVA analysis, adjusting for baseline value

Mean scores for the seven individual PoSSe subscales were similar in both groups, with no statistical significance observed individually or overall. Figure 47 provides a graphical representation of the similarities between both study groups.
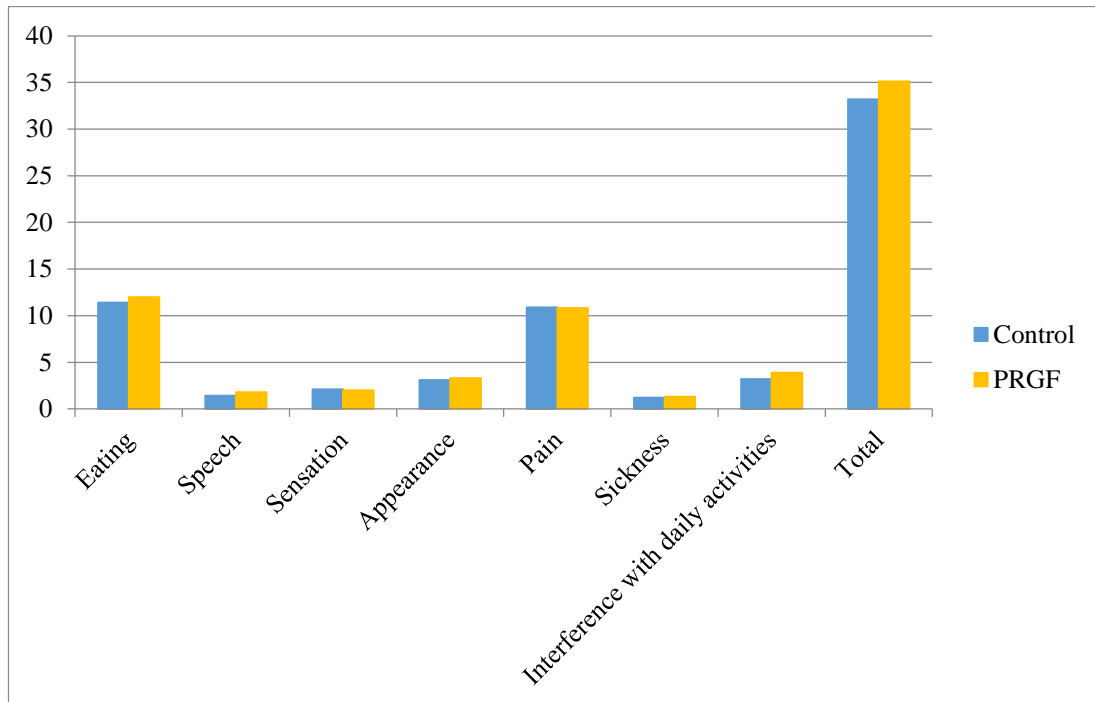
Figure 47. Bar chart representation of mean PoSSe subscale scores and mean total PoSSe scores in control and PRGF groups. X-axis, PoSSe subscale; y-axis, PoSSe score

4.10 SECONDARY OUTCOME MEASURES AT DAY SEVEN

4.10.1 Mouth opening

Postoperative mouth opening (MIO) was recorded for 63 patients at T2. The remaining 11 patients failed to return for follow-up. On average, mouth opening reduced by almost 19% in both study groups between T0 and T2 with no statistical difference found between groups (p=0.67). Mean T2 MIO was calculated at 35.7mm (SD=8.2) for the control group and 35.4mm (SD=8.5) for the PRGF group. Baseline and T2 MIO data are summarised in Table 34.

4.10.2 Periodontal probing depth

Mean periodontal probing depths (PPD) were identical in both groups at T0 and T2 with no statistical significance observed (p=0.91). PPD increased on average by 3.1mm between T0 and T2. Baseline and T2 PPD data are summarised in Table 34.

Outcome	Treatme nt	n	Baseline Mean ± SD	7-days post Mean ± SD	Group Difference [*] Mean (95% CI)	P-value
Mouth opening (mm)	Control	32	42.5 ± 6.7	35.7 ± 8.2	0	0.67
	PRGF	31	43.5 ± 7.2	35.4 ± 8.5	-0.8 (-4.7, 3.0)	
Periodontal probing depth (mm)	Control	32	4.1 ± 1.1	7.2 ± 1.9	0	0.91
	PRGF	31	4.1 ± 1.5	7.2 ± 2.2	-0.1 (-1.1, 0.9)	

Table 34. Secondary outcome variables (mouth opening, periodontal probing depth) at postoperative day 7
(*) Calculated from ANCOVA analysis, adjusting for baseline value

4.10.3 Alveolar osteitis

The presence of alveolar osteitis (AO) was documented where signs and symptoms of Blum's criteria were observed and reported (section 2.3.6). Four patients in total developed AO postoperatively, 1 in the control group (3%) and 3 in the PRGF group (9%). This outcome was not found to be of statistical significance (p=0.3).

4.10.4 Socket healing

Socket healing was recorded at T2 by a single clinician using the modified Landry et al healing index (section 3.2.4.1.4). Socket healing was scored on a 7-point scale with 0 indicating poor healing, and 7 excellent healing. Mean healing scores were 4.0 (SD = 1.2) in the control group and 3.6 (SD = 1.2) in the PRGF group. Healing was not found to vary significantly between the two groups (p=0.21).

4.10.5 Analgesia consumption

Data obtained with respect to postoperative analgesia consumption did not follow a normal distribution and statistical analysis was performed using the non-parametric Mann-Whitney test. There was a reduction in reported analgesia consumption in both groups after T1. No statistical difference was found between the groups with respect to paracetamol (p=0.63), ibuprofen (p=0.46) and codeine (p=0.89) consumption between T1 and T2.

4.10.6 Days off work

This outcome variable followed a skewed distribution, with a median of 0 [IQR 0, 2] days off work recorded for patients in the control group, excluding the day of surgery (T0), compared to a median of 2 [IQR 0, 2] lost work days in the PRGF group. These findings did not reach statistical significance (p=0.09).

A summary of these secondary outcome variables is presented in Table 35.

Outcome	Control n n (%)		PRGF n n (%)		Difference [*] RR (95% CI)	P-Value
Dry socket	33	1 (3%)	33	3 (9%)	3.00 (0.33, 27.4)	0.30
Outcome	**Control** n Mean ± SD		**PRGF** n Value		**Difference [**]** Mean (95% CI)	**P-Value**
Landry index	31	4.0 ± 1.2	32	3.6 ± 1.2	-0.4 (-1.0, 0.2)	0.21
Outcome	**Control** n Median [IQR]		**PRGF** n Median [IQR]		**Difference [**]** Median (95% CI)	**P-Value**
Paracetamol	33	12 [0, 22]	33	8 [1, 22]	0 (-8, 3)	0.63
Ibuprofen	33	6 [0, 11]	32	8 [2.5, 11.5]	1 (-2, 4)	0.46
Codeine	33	2 [0, 7]	33	1 [0, 7]	0 (-2, 1)	0.89
Days off work	27	0 (0, 2)	24	2 (0, 2)	1 (0, 2)	0.09

Table 35. Secondary outcome variables (dry socket, socket healing, analgesia consumption and days off work) at postoperative day 7
(*) Risk Ratio calculated as PRGF divided by Control
(**) Difference calculated as PRGF minus Control

CHAPTER FIVE

DISCUSSION

5.1 STUDY DESIGN

This study took the form of a prospective randomised controlled clinical trial. Randomised controlled trials (RCTs) are *"the workhorse of evidence-based healthcare and the only research design that can demonstrate causality, that is, that an intervention causes a direct change in a clinical outcome"* (Brocklehurst and Hoare, 2017). Figure 48 summarises the indications and properties of the different study types.

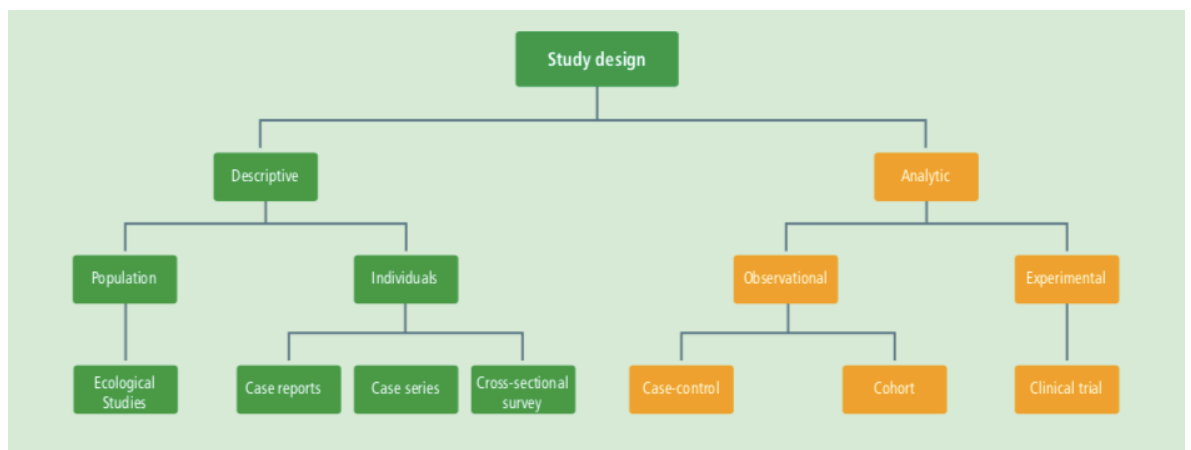**Figure 48.** Trials in context (Brocklehurst and Hoare, 2017)

The bidirectional hypothesis under investigation in this study is whether adjunctive use of PRGF in mandibular third molar (M3M) extraction sockets is superior, equivalent or inferior, to the standard M3M removal technique; this study is thus a head-to-head or superiority trial, and is the study type of choice for our research question.

5.1.1 Parallel group design

In clinical research, a parallel-group method where groups are individually randomised is generally favoured as the standard RCT design (Brocklehurst and Hoare, 2017). In contrast, a split-mouth design tends to be seen more frequently in third molar interventional studies where patients act as their own controls (Ritto et al., 2019, Lima et al., 2018, Armond et al., 2019, Kaewkumnert et al., 2020, Özveri Koyuncu et al., 2020, Eshghpour et al., 2018, Albuquerque et al., 2017, Barbalho et al., 2017, Silva de Oliveira et al., 2016, Glória et al., 2020, Degala and Bathija, 2018, Kim et al., 2020). Authors of the 2020 Cochrane Review 'Surgical techniques for the removal of mandibular wisdom teeth' included 62 RCTs in their analysis, 38 (61%) of which adopted a split-mouth design (Bailey et al., 2020). A separate recently published narrative review investigating CROMs reporting in M3M research found a similar proportion (60%) of third molar studies favouring a split-mouth design (O'Sullivan and Ní Ríordáin, 2021).

Split-mouth studies have the distinct advantage of reducing sample size as well as allowing direct comparisons to be drawn between the 'intervention' and 'control' sides of the mouth. However, they are not without their limitations:

- Lack of stratification e.g. gender, age
- Restriction of outcome measure selection e.g. mouth opening measurement
- Potential for error in the case of patient-reported outcomes
- Unsuitable for quality of life evaluation

These limitations were overcome in our study by adopting a parallel-group design which permitted greater flexibility in PROMs and CROMs selection.

5.2 STRATIFIED RANDOMISATION

There was a strong female preponderance among our study population (77%), and this imbalance was managed by successfully stratifying for gender at the outset. It has long been reported that pain experience is largely influenced by gender, with females tending to report higher levels of postoperative pain. In their cohort of 255 patients undergoing surgical removal of a unilateral M3M under local anaesthesia, Grossi et al (2007) found that females were twice as likely as males to experience severe postoperative discomfort.

Factors such as impaction type and smoking status have also been shown to influence postoperative morbidity following third molar surgery, with distoangular and horizontal impaction types being the biggest offenders as well as current smoking habit (Bello et al., 2011). In our cohort, an equal number of distoangular impactions was observed in both study groups (n=6 in each group), while 9 patients (25%) in the PRGF group had horizontal third molar impactions compared to 6 (15.8%) in the control group. It is unclear whether this marginal imbalance had any tangible influence on postoperative outcomes in the PRGF group. Meanwhile, there were 11 smokers in the control group (28.9%) and 12 in the PRGF group (33.3%).

Overall, both study groups were well balanced with respect to baseline demographics and clinical characteristics, as demonstrated in Table 36.

Variable	Category	Control (n=38)	PRGF (n=36)
Age	-	29.4 ± 6.5	26.8 ± 4.5
Gender	Female	29 (76%)	28 (78%)
	Male	9 (24%)	8 (22%)
Ethnicity	Irish	34 (89%)	32 (89%)
	British	2 (5%)	0 (0%)
	Other	2 (5%)	4 (11%)
Current smoker	No	27 (71%)	24 (67%)
	Yes	11 (29%)	12 (33%)
Surgical time (mins)	-	13.4 [7.6, 19.7]	12.9 [9.2, 16.0]
ASA score	1	20 (53%)	21 (58%)
	2	18 (47%)	15 (42%)
Type of impaction	Distoangular	6 (16%)	6 (17%)
	Mesioangular	11 (29%)	8 (22%)
	Vertical	15 (39%)	13 (36%)
	Horizontal	6 (16%)	9 (25%)
Pederson score	4	6 (16%)	3 (8%)
	5	13 (34%)	14 (39%)
	6	10 (26%)	12 (33%)
	7	6 (16%)	6 (17%)
	8	3 (8%)	1 (3%)
Tooth sectioning	No	16 (42%)	19 (53%)
	Yes	22 (58%)	17 (47%)
Bone removal	No	15 (39%)	16 (44%)
	Yes	23 (61%)	20 (56%)

Table 36. Demographic and surgical characteristics
Summary statistics are: mean ± standard deviation, median [inter-quartile range] or number (percentage)

5.3 STUDY POPULATION AGE

The mean age of our study population (28.1years, range 19-39) reflects the prolonged retention of impacted third molars that is widely known to be directly linked to publication of the 2000 NICE document 'Guidance on the Extraction of Wisdom Teeth' (NICE, 2015). This document resulted in a demonstrable change in practice both in the UK and in Ireland, with clinicians shifting towards retention of symptom-free impacted third molars. In one nationwide review of secondary care admissions in the UK for third molar removal between 1989 and 2009, the authors reported a dramatic increase in the mean age of referred patients from 25years in 1989 to 32years in 2009, with a modal age of 29 years (McArdle and Renton, 2012). It is now widely established that prolonged retention of mesioangular and horizontal mandibular third molars carries a significant risk of distal cervical caries in the adjacent second molar, which is often detected at a very late stage leading to further unnecessary tooth loss (McArdle et al., 2018a).

Many third molar studies report strict age criteria for study participants, with many authors declaring 35years as the upper age limit (Gupta and Agarwal, 2020, Eshghpour et al., 2014, Ogundipe et al., 2011, Varghese et al., 2017, Atalay et al., 2020, Silva de Oliveira et al., 2016, Lima et al., 2018) and more still capping the age limit as low as 30years (Kaewkumnert et al., 2020, Ritto et al., 2019). In setting the upper age limit of our study population at 40years, we endeavoured to collect real-world effectiveness data that would be applicable to the wider target population. A similar approach has been adopted by other third molar researchers

(Lau et al., 2021, Kaplan and Eroğlu, 2016, Degala and Bathija, 2018, Lim and Ngeow, 2017).

With NICE Third Molar Guidance currently under review and following the recent update to RCS Eng guidance, it is likely the landscape will change further in the coming years with respect to population age. A desirable outcome would be a set of evidence-based recommendations for third molar surgery that has garnered agreed consensus from relevant stakeholders and service providers.

5.4 PRGF USE IN THIRD MOLAR SURGERY

There is a paucity of published literature on the topic of PRGF use in third molar surgery. A literature search of the PubMed and EMBASE databases conducted in March 2021 using the search terms "PRGF AND third molar", "PRGF AND wisdom teeth", "plasma rich in growth factors AND third molar" and "plasma rich in growth factors AND wisdom teeth" generated only 5 articles, 3 of which were RCTs (Haraji et al., 2012, Mozzati et al., 2010, Huchim-Chablé et al., 2021), all adopting a split-mouth design. Haraji et al investigated the incidence of AO as their primary outcome measure, but the authors did not disclose their diagnostic criteria. The study by Huchim-Chable is tainted by observation bias, with all study participants (n=10) receiving PRGF in the right-side M3M socket, with the left-side socket acting as the control in all cases. None of the three studies conducted a sample size calculation and are likely underpowered. The quality of the current body of literature on this topic is at best questionable.

5.5 PRGF AND QUALITY OF LIFE

A similar literature search was conducted to explore quality of life (QoL) reporting with PRGF treatment. Of the 10 articles generated, two looked specifically at dental extractions (King et al., 2018, Shah and Cairns, 2018). King et al carried out a clinical trial to compare pain scores and clinical outcomes (halitosis, dysgeusia, exposed bone, inflammation) in 38 patients with 44 sockets who received treatment for post-extraction AO with either PRGF (n=22) or conventional alveogyl dressing (n=22). The authors failed to implement valid instruments for evaluation of QoL outcomes, instead listing generic outcomes such as swelling, pain, bruising, bleeding and healing as QoL parameters. A further weakness of this study is the failure to blind study participants. Unfortunately, the full-text article by Shah and Cairns was not accessible and only the abstract could be reviewed. The authors conducted a systematic review of all autologous platelet concentrates (PRP, PRGF, L-PRF) on M3M socket healing. They concluded APC use improves soft tissue healing, PPD distal to the neighbouring tooth and bone density and reduces postoperative swelling and trismus. No advantage was observed with respect to development of AO or postoperative pain with adjunctive use of APCs. The authors cite study heterogeneity as a barrier to data merging for meta-analysis.

In contrast, we utilised two instruments with a long track record of validity and sensitivity to fully evaluate what we believe are important yet under-reported QoL outcomes in our target population. Our results corroborate previous reports of a notable deterioration in QoL for patients up to one week following M3M removal

(Ogden, 2014). Median OHIP-14 scores in our cohort were highest at T2 in both study groups. OHIP-14 scores increased from a median of 15 [IQR 8, 20] at baseline, to 16 [IQR 5, 20] at T1 and 19.5 [IQR 8, 28] at T2 in the control group, with similar trends observed in the PRGF group at baseline [8.5, IQR 4.5, 15,5], T1 [15, IQR 8, 28] and T2 [16.5, IQR 4.5, 29]. An earlier study evaluating QoL outcomes in 93 patients undergoing M3M removal under local anaesthesia using the OHIP-14 instrument found QoL outcomes deteriorated for 5 days postoperatively, with scores returning to baseline levels by day 7 (McGrath et al., 2003a). The reasons for this disparity are unclear, but may be due to a lower mean study population age in the latter (26years, SD=8) compared to our mean of 28.1years (SD=5.8), as well as racial differences between study populations; 89% of our study population were Irish Caucasian, while McGrath et al conducted their study on an Asian population in Hong Kong. Further research is merited to fully evaluate potential racial differences in perceived QoL outcomes following third molar surgery.

Additionally, PoSSe total scores followed similar trends at each timepoint in our cohort. Mean PoSSe scores in the control group increased from 9% (SD=10.9) at baseline to 26.2% at T1 (SD=10.9) and to 33.2% (SD=15.5) at T2, while in the PRGF group, mean scores similarly increased from 11.4% (SD=12.2) at baseline to 30.3% (SD=10.0) and again to 35.1% (SD=15.0) at T2. Total T2 PoSSe scores in our cohort are similar to those cited in a similar study investigating QoL outcomes in patients undergoing M3M surgical removal under local anaesthesia (Grossi et al., 2007) with authors reporting a mean PoSSe score of 35.7% (SD=13.52).

5.5.1 Quality of life domains

The researchers responsible for development of the PoSSe instrument have previously cited the 'pain' subscale and the total PoSSe score as being most responsive to change (Ruta et al., 2000). Indeed in our cohort, scores for the 'pain' subscale reflect the trends in NRS pain score, the former scoring 7.6% (SD=2.7) at T1 and 10.9% (SD=4.1) at T2 in the control group and 7.8% (SD=3.2) at T1 and 10.8% (SD=4.1) at T2 in the PRGF group. Both study groups demonstrated higher 'pain' subscale scores at T1 and again at T2, indicating a deterioration in pain experience from day 3 to day 7 postoperatively. Similar findings were observed in the 'physical pain' domain of the OHIP-14 which scored most highly in both study groups at each timepoint.

Furthermore, the 'eating' subscale generated the highest scores of all PoSSe subscales in both our study groups, with scores of 9.4% (SD=4.4) and 11.2% (SD=3.8) in control and PRGF groups at T1 respectively, increasing further to 11.4% (SD=5.8) and 12.0% (SD=5.7) at T2. The 'eating' subscale records patient perceptions of mouth opening ability and their enjoyment of food, which in our cohort progressively worsened by T2. These T2 scores are mirrored in the clinical outcome measure of mouth opening (MIO), with our control group demonstrating a 16% reduction in MIO between baseline and T2 and the PRGF group experiencing 18.6% reduction. The impact of eating on QoL is not a newly recognised phenomenon (Savin and Ogden, 1997), but should form an integral part of the informed consent process. With evidence clearly demonstrating an impact on dietary selection and chewing ability one week following third molar surgery,

it follows that patients should be informed of such as part of the decision-making process.

5.6 COVID-19 PANDEMIC

Clinical trials are not without their obstacles, and it is no exaggeration to say that the global Covid-19 pandemic brought with it a unique set of challenges. Data collection for our study ground to a halt in March 2020 following declaration of a global pandemic by the World Health Organisation (Figs 35 & 49) and subsequent cessation of clinical activity at Cork University Dental Hospital with immediate effect. Despite appreciable progress with recruitment and data collection up to that point, public confidence had suffered a distinct setback at the point of resumption of clinical activity in September 2020. A tangible caution regarding the risks associated with aerosol-generating procedures (AGPs) coupled with a reluctance to travel beyond government-enforced restrictions, had a knock-on effect on the recruitment process. Additionally, patient footfall through the hospital was substantially reduced to comply with social distancing in patient waiting areas, and to allow sufficient fallow time in each dental surgery between AGPs (Scottish Dental Clinical Effectivenss Programme, 2021).

A further consequence of the Covid-19 pandemic was the higher than anticipated attrition rate of 14.9% in our study, with 11 patients failing to return for follow-up. Development of Covid-19 symptoms during the immediate postoperative week and close contact with Covid-positive cases accounted for some of our losses to follow-up. Efforts to mitigate these losses were made by telephoning

the relevant patients at postoperative day 7 (T2) and documenting OHIP-14, PoSSe and NRS pain data where contact was successful. In so doing, PROMs data were collected for the target sample of 66 patients.
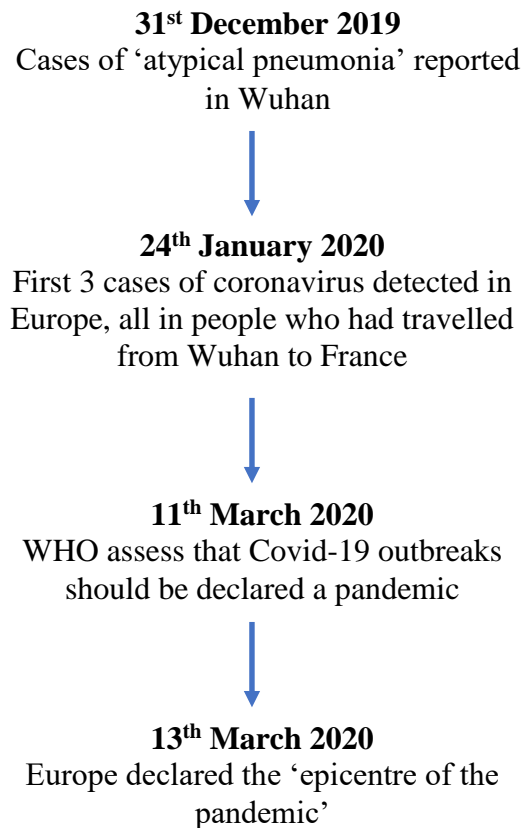
31st December 2019
Cases of 'atypical pneumonia' reported in Wuhan

↓

24th January 2020
First 3 cases of coronavirus detected in Europe, all in people who had travelled from Wuhan to France

↓

11th March 2020
WHO assess that Covid-19 outbreaks should be declared a pandemic

↓

13th March 2020
Europe declared the 'epicentre of the pandemic'

Figure 49. Timeline of WHO response to Covid-19 (available at https://www.who.int/news/item/29-06-2020-covidtimeline)

5.7 STUDY STRENGTHS

Our study was conducted as a prospective RCT, which is the gold standard for interventional clinical trials and was the chosen study type for data analysis in the aforementioned recent Cochrane Review on 'Surgical techniques for the removal of mandibular wisdom teeth' (Bailey et al., 2020).

5.7.1 Blinding

One of the strengths of our study is the robustness of the blinding methodology. At no point throughout the course of the study were patients or the lead investigator unblinded. This sets our study apart from similar previously published studies adopting a parallel-group design where blood was obtained only from patients allocated to the experimental arm of the study (Anitua et al., 2015a, Ogundipe et al., 2011) or where patient blinding was unclear (Jeyaraj and Chakranarayan, 2018, Dutta et al., 2015). Failure to ensure patient blinding will inevitably lead to detection bias and ultimately call into question the reliability and generalisability of results.

5.7.2 Single observer

A single clinician took responsibility for all data collection, CROMs measurement, PROMs documentation and virtual telephone reviews for the purposes of this study. Allocation of these roles to one person eliminated the need for observer calibration, thereby ensuring consistency and accuracy of the data and is a further strength of our methodology.

5.7.3 Scope of outcome measures

To our knowledge, no other study has utilised validated instruments to explore patient-reported as well as clinician-reported outcome measures in third molar interventional RCTs utilising autologous platelet concentrates. There appears to

be a trend for reporting either CROMs or PROMs (with the exception of pain score) in third molar trials, but never both, and the reasons for this trend are unclear.

For instance, Ritto et al (2019) conducted a split-mouth study (n=20) to investigate the effects of L-PRF on third molar socket healing and interproximal periodontal pocket depth; Afat et al (2019) compared L-PRF alone versus L-PRF with HA (hyaluronic acid) to placebo in their parallel-group study, reporting soft tissue healing, infection, AO and haemorrhage as outcome measures; Ozveri-Koyuncu (2020) in turn investigated the effects of CGF (concentrated growth factor) on soft tissue healing, swelling and mouth opening following third molar surgery.

Although PROMs are justifiably lauded for measuring data that are pertinent to the patient rather than the disease or intervention, it should be noted that they are not a replacement for objective clinical outcome measures (Higginson and Carr, 2001). Instead PROMs and CROMs should be considered complementary tools. Our aim was to demonstrate this synergism by selecting a core set of CROMs and PROMs that are easily measurable, reproducible and valid in our study and target populations.

Mouth opening (MIO) and swelling measurement are by far the most commonly reported CROMs in third molar studies (O'Sullivan and Ní Ríordáin, 2021), but the latter has been criticised for its lack of standardisation (Coulthard et al, 2014). We successfully demonstrated the direct relationship between postoperative reduction in MIO and higher scores in the PoSSe 'eating' subscale in both study

groups. We are confident in our MIO measurements due to its reproducibility, and the fact that all measurements were recorded by a single clinician.

Similarly, selection of both an oral health-related instrument as well as a third molar-specific instrument for QoL evaluation adds value to the results. The internal consistency demonstrated by the PoSSe instrument makes it particularly useful for group comparisons in patients undergoing the same procedure (Ruta et al., 2000). Moreover, the OHIP-14 and PoSSe instruments appeared to demonstrate agreement in pain detection with the 'physical pain'/'pain' subscales both scoring highly at T1 and T2 in both study groups.

5.8 STUDY LIMITATIONS

5.8.1 Follow-up intervals

The changes in life quality experienced during the first postoperative week after third molar surgery are well documented (Savin and Ogden, 1997, Ogden, 2014, McGrath et al., 2003a). McGrath et al captured QoL data daily for 7 postoperative days in their cohort of 93 patients. They were able to demonstrate a sharp decline in life quality on postoperative day 1, followed by a more gradual deterioration up to and including day 5, after which QoL was shown to improve and return to baseline by day 7.

While we would have liked to have been able to capture QoL trends in as much detail, our focus on CROMs as well as PROMs rendered this an unfeasible task.

We believed a choice had to be made to either invite patients back to the clinic for follow-up, or provide each patient with a questionnaire pack to complete during the first postoperative week. We selected the former option to optimise completeness and accuracy of the resulting data. Our experience was that those patients who did attend at T2 for postoperative review felt reassured for having done so.

Ideally, we would have liked to assess patients physically at T1 rather than virtually, but we felt that mandating two postoperative clinic visits would render recruitment challenging and result in significantly higher rates of attrition. Factors such as long travel distances, childcare barriers and time off work influenced this decision. We still collected comprehensive PROMs data for our patients at T1, and felt this was a worthy compromise for the greater goal of satisfactory attendance at T2. In hindsight, the virtual review decision was a fortuitous one considering the Covid-19 pandemic that unfolded mid-study.

5.8.2 OHIP-14 binary data

Dichotomisation of the OHIP-14 data collected in our study was performed for ease of analysis. In many instances, the non-discriminatory nature of the responses 'never', 'hardly ever', 'occasionally', 'fairly often' and 'very often' resulted in more than one response being ticked on the questionnaire for a single question. We subsequently decided to merge the negative responses 'never' and 'hardly ever' as a 'no' and 'occasionally', 'fairly often' and 'very often' as a 'yes'.

Typically, OHIP-14 total scores are on a scale of 0 to 56, with 56 indicating the most significant impact on oral health-related life quality.

However, despite this limitation, we were able to demonstrate commonality between PoSSe data and CROMs data in both groups and it is unlikely that dichotomisation of our OHIP-14 data had any demonstrable impact on our overall results.

5.8.3 PoSSe data

The PoSSe instrument is not validated for use as a preoperative tool (Ruta et al., 2000), and while we recognise this as a limitation, we felt it was important to capture PoSSe data at T0 and T1 along with the other outcome variables, to permit full evaluation of trends in QoL arising as a result of the intervention. We believe collection of baseline and T1 PoSSe data serves to strengthen rather than detract from our overall results.

5.8.4 Periodontal probing depth

In hindsight, periodontal probing depth (PPD) yields more value as a screening tool as opposed to a useful day 7 CROM. Demonstration of increased interproximal PPD is one of the many indications for removal of M3M, and evaluation of the periodontal health of the neighbouring molar tooth following M3M removal is a useful exercise. However, its relevance 7 days post-extraction is limited, and PPD evaluation would be better suited to evaluation several weeks

or months postoperatively once healing is fully complete (Kim et al., 2020, Ritto et al., 2019). Nonetheless, it is useful to have this periodontal information for the mesioangular and horizontal impactions included in our study in view of the NICE guidance updates currently in progress.

5.8.5 Loss to follow-up

We allowed for an anticipated 12% dropout rate, and set our target sample size at 74 patients. Unfortunately, loss of 11 patients to follow-up at T2 resulted in a higher than anticipated 15% attrition rate which leaves our study slightly underpowered (n=63).

To mitigate these losses, the same single clinician responsible for all virtual review appointments telephoned those 11 patients who failed to return for T2 follow-up on the day in question and was successful in reaching 3 of these patients. This enabled documentation of OHIP-14, PoSSe and NRS pain data for same. Hence our target sample of 66 patients was met with respect to PROMs data.

5.9 RECOMMENDATIONS FOR FUTURE RESEARCH

We have conducted a robust randomised controlled clinical trial exploring the previously untapped subject of QoL outcomes with adjunctive use of PRGF in third molar sockets. Such is the robustness of our methodology, that it would provide a useful template for interested researchers looking to conduct a similar study.

Although our study failed to demonstrate any quantifiable clinical or QoL advantage for patients undergoing M3M removal with adjunctive PRGF, there is undoubtedly scope to apply a similar methodology to other patient groups and research questions.

For instance, the surgical techniques explored in the 2020 Cochrane Review (Bailey et al., 2020) such as flap type, lingual nerve protection, bone removal technique, wound irrigation technique and primary versus secondary wound closure could certainly be investigated by adapting our methodology.

It would also be worthwhile to investigate QoL outcomes with use of PRGF in non-third molar patient cohorts. Evaluation of the effectiveness of PRGF as a socket preservation technique prior to dental implant placement would be a worthwhile research topic, although patient recruitment might be complicated by factors such as a diverse target population with inherent heterogeneity and challenges in matching surgical difficulty of the extractions.

MRONJ is a topic with which all oral surgeons are familiar, and which shows a distinct lack of high-quality research. A recently published review paper demonstrated a clear lack of QoL data in this patient cohort, particularly oral health-related (Murphy and Mannion, 2020). A heterogenous patient population with multiple comorbidities would similarly challenge patient selection. Nonetheless, in order to inform future practice and to truly evaluate the course of this chronic disease, accepting the inherent challenges may be necessary in order

to collect meaningful effectiveness data with respect to MRONJ management with

PRGF and associated impact on QoL.

CHAPTER SIX

CONCLUSION

6.0 CONCLUSION

This study failed to show any statistically significant differences in patient-reported and clinician-reported outcomes in patients who underwent adjunctive socket treatment with PRGF compared to conventional third molar surgical removal, and the null hypothesis was thus accepted.

Marginal variations in NRS pain scores were not significant between study groups at any timepoint (T1 p=0.06; T2 p=0.44). Pain scores were at worst on the borderline of mild to moderate, with the highest mean NRS pain scores recorded 3 days postoperatively in the PRGF group (NRS=4.1, SD=2.4). The age old adage that cure is often worse than the 'disease' itself is reflected in the NRS pain trends during the study period. These observations are helpful in educating patients regarding appropriate pain management during the first 3 postoperative days in particular.

OHIP-14 scores and PoSSe subscale and total scores were also found not to vary significantly between study groups in this instance. Of note is the impact of third molar removal on chewing ability as a consequence of reduced mouth opening. Of all PoSSe subscales, 'eating' ranked highest in terms of impact on life quality at both days 3 and 7 postoperatively, correlating with objective mouth opening measurements at T2 which were on average 16% and 18.6% less than baseline measurements in the control and PRGF groups, respectively. With this in mind, it would be advisable for clinicians to discuss such functional limitations with

prospective patients during the treatment planning process to ensure adequate informed consent.

Although this study failed to demonstrate superiority in performance of adjunctive PRGF therapy over conventional M3M surgery, there is no disputing the body of evidence in support of its physiological advantages. Ours is one of the few RCTs to utilise PRGF over the more widely available L-PRF in this study population. The latter formulation boasts a considerable catalogue of publications declaring its therapeutic advantages, although the quality of available studies has not been deemed sufficiently high to draw any solid conclusions with regard to L-PRF use in third molar surgery (Bailey et al., 2020). Much of the evidence should therefore be approached with caution.

It is the authors' opinion that this study sets a benchmark for future similar RCTs due to the reproducibility of the methodology, and it is hoped that it will encourage further research on the topic of quality of life in third molar clinical trials. The responsiveness of the PoSSe and OHIP-14 instruments in this patient cohort also supports their application as clinical audit tools to assess surgical outcomes.

Despite the statistically insignificant conclusions drawn in this study, it is the authors' firm conviction that PRGF has an important role to play in oral surgery, and it is worth exploring quality of life outcomes in other patient cohorts.

CHAPTER SEVEN

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CHAPTER EIGHT

APPENDICES

APPENDIX A – BAOS Presentation certificate

The British Association of Oral Surgeons

This is to certify that

LAURA O'SULLIVAN

Presented an Open Paper at the online BAOS Annual Award Ceremony Open Paper Presentations on **Thursday 18 March 2021** on the topic of

PRGF® Use in Third Molar Surgery: a Randomised Controlled Trial

Organised according to the standard established by

The British Association of Oral Surgeons

Signed ....

Professor Paul Coulthard: - President, British Association of Oral Surgeons

This is a certificate of participation and does not infer ability to practice. It may not be used to indicate a Professional qualification of a license to perform a specific procedure. Individual accountability for any action subsequently taken by the holder must be governed according to his or her particular professional rules of codes of practice.

The abstract of this poster will be published in the BAOS Journal.

The British Association of Oral Surgeons

Promoting excellence in Oral Surgery through education, training and research for better patient care.

APPENDIX B – EACMFS Presentation certificate

25th EACMFS CONGRESS
14-16 JULY 2021
VIRTUAL EVENT

The Scientific Committee of the 25th Congress of the European Association for Cranio Maxillo Facial Surgery virtual congress, held in July 14-16, 2021

CERTIFIES THAT

the following oral paper has been accepted and presented at the Congress:

Investigation of the effect of plasma rich in growth factors on quality of life following mandibular third molar removal: a randomised controlled clinical trial
Laura Maria O'Sullivan, Rícheal Ní Ríordáin

Cork University Dental School and Hospital, Cork, Ireland

Prof. Jean-Paul Meningaud
President EACMFS 2021

Prof. Joel Ferri
Scientific Director

APPENDIX C – Publication tracker The Surgeon journal

Track Your Accepted Article

The easiest way to check the publication status of your accepted article

Variations in reporting of clinician-reported outcome measures in third molar surgery: a focused review

Article reference	SURGE918
Journal	The Surgeon
Corresponding author	Laura O'Sullivan
First author	Laura O'Sullivan
Received at Editorial Office	11 Nov 2020
Article revised	6 Mar 2021
Article accepted for publication	25 Mar 2021

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Last update: 4 May 2021   ✉ Share via email

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- Your article has been received for production.
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Production events

Bibliographic information

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Will appear soon

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Will appear soon

APPENDIX D – Publication acceptance email OOOO journal

Date:	26 Apr 2021
To:	"Laura Maria O'Sullivan" laura.osullivan@ucc.ie
From:	"OOOO (Triple O) journal" tripleojournal@gmail.com
Subject:	Regarding your submission TRIPLEO-D-21-00370: Revision requested

Ms. Ref. No.: TRIPLEO-D-21-00370
Title: Autologous platelet concentrates in oral surgery: protocols, properties and clinical applications
Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology

Dear Dr. O'Sullivan,

I am writing concerning your paper, "Autologous platelet concentrates in oral surgery: protocols, properties and clinical applications", which you recently submitted to Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. A panel of anonymous experts has carefully reviewed your paper. They have suggested some relatively minor recommendations for revision of your paper. The comments are included below.

If you can resolve the issues raised, we look forward to receiving a revised manuscript by 10 Jun 2021 for further consideration. If you cannot submit your revision by this date or choose not to submit a revision, please contact us. Depending on the nature and extent of your revisions, the manuscript may be re-reviewed at that time. We cannot guarantee, even with revision, that the manuscript will achieve a high enough priority for publication. The revision must be accompanied by a separate letter outlining in detail the changes made in the manuscript, referring point-by-point to the questions/criticisms of each reviewer and the editors.

Along with the separate point-by-point description of changes made according to the reviewers' and editors' comments, the revised manuscript must show in HIGHLIGHTED font the revisions made.

To submit a revision, go to https://www.editorialmanager.com/tripleo/ and log in as an Author: Your username is: ********

On the Main Menu page, you will see a menu item called Submission Needing Revision; you will find your submission record there and be able to submit your revision.

Thank you for considering Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology for publication of your work.

I look forward to the early submission of your revised manuscript.

Sincerely,

Antonia Kolokythas, DDS, MSc, MSed, FACS
Editor, Oral & Maxillofacial Surgery
Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology
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APPENDIX E - Endoret® preparation protocol

Available to download at http://bti-biotechnologyinstitute.com/us/bti-channel/2015-us-endoret-prgf-application-musculoskeletal-system/

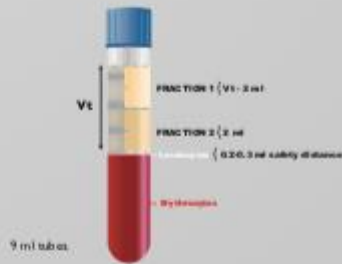endoret (prgf)
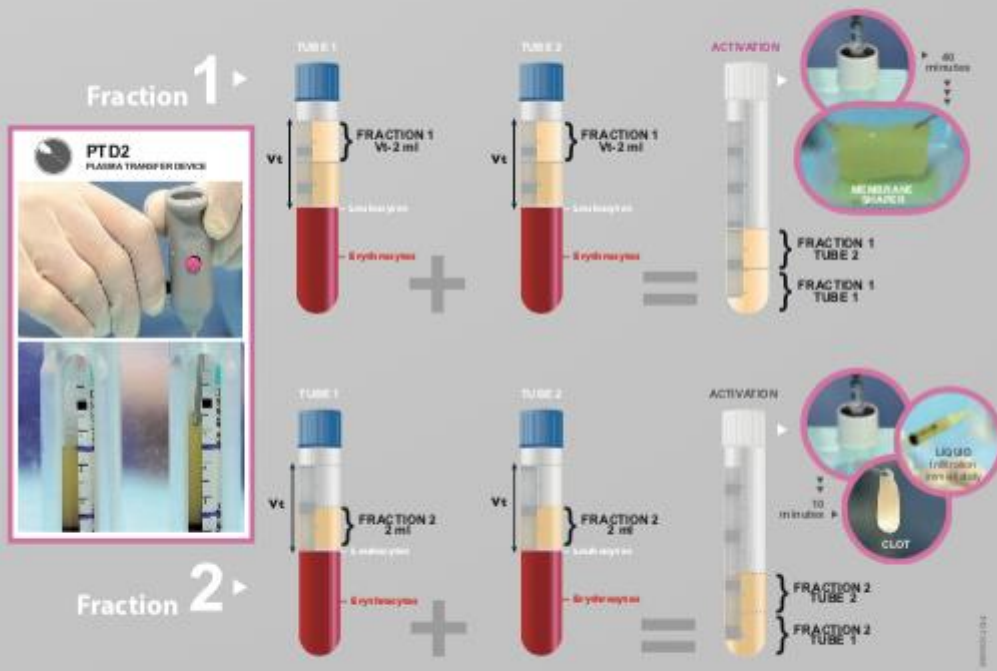
APPLICATION IN THE MUSCULOSKELETAL SYSTEM

FOR APPLICATION USE THE **KIT EDK 1 OR EDK 2** INTENDED FOR THIS FIELD OF MEDICINE.

APPENDIX F – OHIP-14 questionnaire

	OHIP – 14*	
Dimensions	**Questions**	
Functional Limitations	Have you had trouble *pronouncing any words* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often Have you felt that your *sense of taste* has worsened because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	
Physical pain	Have you had *painful aching* in your mouth? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often Have you found it *uncomfortable to eat any foods* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	
Psychologic al discomfort	Have you been *self-conscious* because of your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often Have you *felt tense* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	
Physical disability	Has your *diet been unsatisfactory* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	

	Have you had to *interrupt meals* because of problems with you teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	
Psychologic al disability	Have you found it *difficult to relax* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often Have you been *a bit embarrassed* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	
Social disability	Have you been *a bit irritable with other people* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often Have you had *difficulty doing your usual jobs* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	
Handicap	Have you *felt that life in general was less satisfying* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often Have you been *totally unable to function* because of problems with your teeth, mouth or dentures? ☐never ☐ hardly ever ☐ occasionally ☐ fairly often ☐ very often	

APPENDIX G – PoSSe scale

The Final PoSSe Scale

1. EATING

a.   In the **last week**, has your operation affected your **enjoyment** of food?
*(Please mark **one** box)*

No, not at all [0]   Yes, a little [5.25]   Yes, very much [10.5]

b.   In the **last week**, for how many days were you unable to **open your mouth** normally because of your operation?
*(Please mark **one** box)*

0 days [0]   1–2 days [2.63]   3–4 days [5.25]   5–6 days [7.88]   7 days [10.5]

2. SPEECH

a.   In the **last week**, for how many days was your **voice** affected because of your operation?
*(Please mark **one** box)*

0 days [0]   1–2 days [1.25]   3–4 days [2.5]   5–6 days [3.75]   7 days [5]

b.   On the **worst day** of the **last week**, how badly was your **speech** affected by your operation?
*(Please mark **one** box)*

Not at all [0]   Slightly [1.25]   Moderately [2.5] Severely [3.75]   Unable to [5]
                                                            speak at all

3. SENSATION

a.   Thinking of the **last week**, for how many days were your lips or tongue feeling **tingling** because of your operation?
*(Please mark **one** box)*

None at all [0]   1–2 days [2]   3–4 days [4]   5–6 days [6]   7 days [8]

b.   Thinking of the **last week**, for how many days were your lips or tongue feeling **numb** because of your operation?
*(Please mark **one** box)*

None at all [0]   1–2 days [2]   3–4 days [4]   5–6 days [6]   7 days [8]

4. APPEARANCE

a.   Thinking of the **last week**, for how many days were your face and/or neck **bruised** because of your operation?
*(Please mark **one** box)*

None at all [0]   1–2 days [1.5]   3–4 days [3]   5–6 days [4.5]   7 days [6]

b.   Thinking of the **last week**, for how many days were your face and/or neck **swollen** because of your operation?
*(Please mark **one** box)*

None at all [0]   1–2 days [1.5]   3–4 days [3]   5–6 days [4.5]   7 days [6]

5. PAIN

a.   Thinking of the **last week**, for how many days did you experience **pain** from your operation?
*(Please mark **one** box)*

None at all [0]   1–2 days [2.38]   3–4 days [4.75]   5–6 days [7.13]   7 days [9.5]

b.   Thinking of the **last week**, has the pain from your operation been controlled by **painkillers**?
*(Please mark **one** box)*

I have had no pain. [0]
Yes, completely controlled. [2.38]
Controlled mostly but still some discomfort. [4.75]
Poorly controlled. [7.13]
Not controlled at all. [9.5]

6. SICKNESS

a.   Thinking of the **last week**, for how many days did you **vomit** or **feel nauseated**?
*(Please mark **one** box)*

None at all [0]   1–2 days [1.25]   3–4 days [2.5]   5–6 days [3.75]   7 days [5]

b.	On the **worse day** of the last week, **how many times** did you vomit or feel nauseated?

*(Please mark **one** box)*

Not at all [0]   One day [1.25]   2–3 times [2.5]   More than [3.75]   All the time [5]
                                                     3 times   (all day long)

7. INTERFERENCE WITH DAILY ACTIVITIES

a. In the **last week**, did the operation prevent you from carrying out **work/housework** and other **daily activities**?

*(Please mark **one** box)*

No, not at all. [0]
I could continue with my work, but my work suffered. [0.83]
Yes, for 1 day. [1.65]
Yes, for 2–6 days. [2.48]
Yes, for 7 days. [3.3]

b. In the **last week**, have your **leisure activities** been affected by your operation? (including sports, hobbies and social life)

*(Please mark **one** box)*

Not affected by the operation. [0]
Mildly affected by the operation. [0.83]
Moderately affected by the operation. [1.65]
Severely affected by the operation. [2.48]
The operation prevented any social life at all. [3.3]

c. Thinking of the **last week**, how badly did the pain affected your **life**?

*(Please mark **one** box)*

Not at all [0]   Slightly [1.1]   Moderately [2.2]   Severely [3.3]

APPENDIX H – Pederson classification

CLASSIFICATION	VALUE
Spatial Relationship	
• Mesioangular	1
• Horizontal/transverse	2
• Vertical	3
• Distoangular	4
Depth	
• Level A: occlusal surface M3M level with, or above, occlusal surface of neighbouring tooth	1
• Level B: occlusal surface M3M coronal to CEJ of neighbouring tooth	2
• Level C: occlusal surface M3M apical to CEJ of neighbouring tooth	3
Ramus relationship/available space	
• Class 1: sufficient space	1
• Class 2: reduced space	2
• Class 3: no space	3
Difficulty index*	
• Very difficult	7-10
• Moderately difficult	5-6
• Slightly difficult	3-4