EFFECTS OF MULTI-WALL CARBON NANOTUBES, PLATELET-RICH PLASMA INJECTION, AND THEIR COMBINATION ON TOOTH RELAPSE FOLLOWING ORTHODONTIC TOOTH MOVEMENT: AN EXPERIMENTAL STUDY IN RABBIT

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by

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LIST OF ABBREVIATIONS

ACD	Activator Chloride
ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance
BMP2	Bone Morphogenic Protein 2
BMP4	Bone Morphogenic Protein 4
BVN	Blood Vessels Number
CBCT	Cone Beam Computed Tomography
CMC	Carboxymethyl Cellulose
CNT	Carbon Nanotubes
CVD	Chemical Vapor Deposition
DMM	Direct Manual Measurement
ECM	Extracellular Matrix
EDV	electrical digital Vernier
FDA	Focal Domains Adhesion
f-MWCNTs	Functionalised MWCNTs
FOV	Field of View
FT-IR	Fourier Transformed Infrared Spectroscopy
GPR-30	G Protein-Coupled Receptor-30
H&E	Haematoxylin and Eosin
HIF-1alpha	Hypoxia-Inducible Factor-1 alpha
IPR	Interproximal Reduction
IVM	Indirect Virtual Measurement
LSCC	Low-Speed Centrifugation Concept
LSD	Least Significant Test
M-CSF	Macrophage Colony Stimulating Factor

MWCNT-COOH	Multi-Walled Carbon Nanotubes-Carboxylic Acid
MWCNTs	Multi-Walled Carbon Nanotubes
NBA	New Bone Area
NSAID	Non-Steroidal Anti-Inflammatory Drug
OAP	Orthodontic Appliance Placement
OBN	Osteoblast Cells Number
OCN	Osteoclast Cells Number
OPG	Osteoprotegerin
ORD	Orthodontic Relapse Distance
OTM	Orthodontic Tooth Movement
OTR	Orthodontic Tooth Relapse
PDGF	Platelet-Derived Growth Factor
PDGFRβ	Platelet-Derived Growth Factor Receptor Beta
PDL	Periodontal Ligament
PERK ½	Phosphorylated Extracellular Signal-Regulated Kinases ½
PRP	Platelet Rich Plasma
PTH	Parathyroid Hormone
RANK	Receptor Activator of Nuclear Factor Kappa-B
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
ROI	Region of Interest
Runx2	Runt-related Transcription Factor 2
SD	Standard Deviation
SERM	Selective Oestrogen Receptor Modulator
SWCNT	Single-Walled Carbon Nanotubes
TBA	Total Bone Area
TMD	Temporomandibular Disturbances
TMJ	Temporomandibular Joint

TNF	Tumour Necrosis Factor
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
VFRs	Vacuum-Formed Retainers
WB	Whole blood
WF	Working Field
α-SMA	Alpha-Smooth Muscle Actinα-SMA

LIST OF APPENDICES

KESAN SUNTIKAN TIUB NANO KARBON BERBILANG DINDING, PLASMA KAYA PLATELET DAN GABUNGANNYA TERHADAP RELAPS GIGI SELEPAS PERGERAKAN GIGI ORTODONTIK: KAJIAN EKSPERIMEN KE ATAS ARNAB

ABSTRAK

Rawatan ortodontik adalah penting untuk meningkatkan kesihatan pergigian dengan membetulkan masalah seperti gigi yang bersesak, gigitan berlebihan, dan ketidaksejajaran. Relaps selepas tamat fasa aplian tetap boleh berlaku walaupun selepas rawatan berjaya. Kajian ini meneliti kesan Tiub Nano Karbon Berbilang Dinding (MWCNTs), Plasma Kaya Platelet (PRP), dan gabungannya terhadap pengulangan ortodontik serta perubahan histomorfometrik selepas pergerakan gigi. Sebanyak 72 arnab albino jantan dewasa yang sihat telah dipilih, dengan 65 arnab melengkapkan kajian selepas mengambil kira kadar keluar sebanyak 10%. Arnab tersebut, berumur antara 20–32 minggu dan berat purata 1770 gram, dipelihara dalam keadaan cahaya dan gelap terkawal di Universiti Thi-Qar, Iraq. Kajian ini melibatkan lima kumpulan: Kumpulan I (kawalan negatif) tanpa intervensi; Kumpulan II (kawalan positif) hanya dengan pemasangan alat ortodontik; Kumpulan III dengan alat ortodontik dan suntikan MWCNT; Kumpulan IV dengan gabungan MWCNTs dan PRP; dan Kumpulan V hanya dengan suntikan PRP. Alat ortodontik yang diubah suai digunakan untuk menghasilkan daya resiprokal sebanyak kira-kira 50 gram bagi mendorong pergerakan gigi. PRP disediakan dengan memusatkan platelet melalui proses sentrifugasi darah yang dikumpulkan, manakala MWCNTs disterilkan dan disebarkan untuk memastikan keberkesanannya. Pengukuran pengulangan dilakukan secara manual dan maya menggunakan Tomografi Berkomputer Sinaran Kon (CBCT), bersama penilaian histomorfometrik struktur pergigian dan tulang. Analisis statistik, termasuk ujian T berpasangan dan ANOVA sehala, dijalankan menggunakan SPSS versi 26 untuk menilai pemboleh ubah seperti perubahan berat badan, ruang yang dicipta, jarak pengulangan, dan ketumpatan tulang. Hasil kajian menunjukkan tiada perbesaan ketara dalam peningkatan berat badan antara kumpulan, dan tiada tanda hipertrofi gingiva atau pendarahan diperhatikan. Walaupun perbesaan ruang yang dicipta di kawasan insisal dan servikal tidak ketara, Kumpulan II menunjukkan jarak pengulangan yang lebih besar pada hari-hari tertentu. Variasi ketara dalam ketumpatan tulang kortikal dan tulang spongiosa diperhatikan, dengan Kumpulan I menunjukkan ketumpatan kortikal tertinggi. Ligamen periodontal (PDL) kelihatan normal dalam semua kumpulan, dan tiada ankylosis dikesan. Resorpsi akar terpencil diperhatikan tetapi tidak khusus kepada mana-mana kumpulan. Pembentukan tulang alveolar paling ketara di kawasan servikal dan berkurang ke arah kawasan apikal. Kumpulan III, IV, dan V menunjukkan pembentukan tulang baru yang lebih besar berbanding Kumpulan II, dengan kumpulan yang dirawat dengan MWCNT (Kumpulan III dan IV) menunjukkan lebih banyak saluran darah di kawasan servikal pada penilaian awal dan hari ke-11. Kumpulan I mempunyai bilangan saluran darah yang lebih sedikit secara keseluruhan, dengan perbesaan ketara di kawasan servikal dan tengah. Kajian ini merumuskan bahawa gabungan MWCNTs dan PRP berkesan dalam mengekalkan jarak antara gigi insisor bawah di kawasan insisal dan servikal selepas aplian ortodontik dikeluarkan.

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ABSTRACT

Orthodontic treatment is crucial for improving dental health by addressing issues like crowded teeth, overbites, and misalignments. Relapse following completion of fixed appliance phase can occurs even after successful treatment. This study examines the effects of Multi-Walled Carbon Nanotubes (MWCNTs), Platelet Rich Plasma (PRP), and their combination on orthodontic relapse and histomorphometric changes following tooth movement. A total of 72 healthy adult male albino rabbits were initially selected, with 65 completing the study after accounting for a 10% dropout rate. The rabbits, aged 20–32 weeks and averaging 1770 grams, were housed under controlled light and dark cycles at the University of Thi-Qar, Iraq. The study involved five groups: Group I (negative control) with no intervention; Group II (positive control) with only orthodontic appliance placement; Group III with orthodontic appliance and MWCNT injection; Group IV with a combination of MWCNTs and PRP; and Group V with PRP injection alone. A modified orthodontic appliance generating a reciprocal force of approximately 50 grams was used to induce tooth movement. PRP was prepared by centrifuging collected blood to concentrate platelets, while MWCNTs were sterilised and dispersed to ensure their functionality. Relapse measurements were performed both manually and virtually using Cone Beam Computed Tomography (CBCT), alongside histomorphometric evaluations of dental and bone structures. Statistical analysis, including paired samples T-tests and one-way

ANOVA, was conducted using SPSS version 26 to assess variables such as weight changes, space created, relapse distances, and bone density. The results revealed no significant differences in weight gain among the groups, with no signs of gingival hypertrophy or bleeding. Although differences in space created in the incisal and cervical regions were not significant, Group II displayed greater relapse distances on specific days. Significant variations in cortical and cancellous bone density were observed, with Group I showing the highest cortical density. The periodontal ligament (PDL) appeared normal across all groups, and no ankylosis was detected. Isolated root resorption was noted but was not specific to any group. Alveolar bone formation was most pronounced in the cervical region and decreased toward the apical region. Groups III, IV, and V demonstrated greater new bone formation compared to Group II, with MWCNT-treated groups (Groups III and IV) showing significantly more blood vessels in the cervical region during early and 11th-day assessments. Group I had fewer blood vessels overall, with significant differences noted in the cervical and middle regions. The study concluded that the combination of MWCNTs and PRP was effective in maintaining the spacing between lower incisors in both incisal and cervical regions after removing the orthodontic appliance.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Orthodontic treatment effectively addresses issues such as crowded, misaligned, and protruding teeth. This process involves the remodelling of alveolar bones and the periodontal ligament (PDL), leading to the desired alignment of teeth. Furthermore, the stability of the teeth is predominantly achieved over time. However, relapse is possible even after successful orthodontic treatment, which can happen for several reasons and at any point. Even though teeth and supporting tissue stabilise with time, the threat of the teeth returning to their original position, "relapse," is still possible (Prameswari et al., 2020).

The tendency of the teeth to return to their former position after tooth movement is known as orthodontic relapse. Orthodontic relapse is a detrimental consequence of orthodontic treatment (Utari et al., 2022). It happens within 12 months and can occur much later in life due to several factors (Chaushu et al., 2022).

According to a study by Littlewood et al. (2017), it is reported that almost 50% of patients experience relapse within a decade. Hence, applying after treatment is essential to achieve stable results in the retention phase. Stability can be affected by periodontium, growth and occlusion, and soft tissue pressure. However, periodontium is the main contributor to relapse. Time is required to reorganise the periodontium once orthodontic appliances are detached. The gingival suprarenal fibre is not attached to the bone, unlike the PDL, and the remodelling speed of the fibres is much lower. After removing orthodontic appliances, restructuring the elastic supracrestal fibres may take one year. As a result, supracrestal fibres will cause relapse after orthodontic treatment (Charavet et al., 2022).

Relapse is possible after orthodontic treatment; hence, justification of retention is essential for post-orthodontic treatment for the following reasons:

i) Due to the unstable position of the teeth post-treatment, the tendency of relapse is urged by the soft tissues.

ii) Changes related to growth can also cause relapse.

iii) Periodontal and gingival structures are causes of relapse by orthodontic tooth movement, and time is required when the appliances are removed from the oral cavity (Malandkar et al., 2019).

Orthodontic relapse due to gingival factor and reassembling of construction of periodontium is unimportant for tooth movement. In order to adapt to the new position, adequate time is required for bone and gingival fibres. Different time rates are employed for different positions of periodontium (Motghare et al., 2022).

Currently, it is a challenge for orthodontists to predict the occurrence of relapse assuredly after orthodontist treatment (Littlewood et al., 2017). Factors mentioned above are more prone to relapse even after providing successful orthodontist treatment to the patients.

Prediction of the formation of relapse helps orthodontists identify the problem and solve the issue in the early stages. Relapse is considered to be a long-term issue. Hence, before undergoing orthodontic treatment, the dentist should advise patients about the consequences of retention and relapse since relapse can occur at any time. Orthodontic relapse is unavoidable; however, it can be reduced or controlled by following certain measures. One such solution is retainers (Arn et al., 2020). There are various types of retainers for various purposes.

Hawley retainers (Alassiry, 2019) are made of acrylic with metal wire. One of the significant advantages of using Hawley retainers is that they are easy to maintain and clean, can be easily repaired if broken, and, most importantly, are cost-effective. However, there are a few disadvantages, including the possibility of losing the appliance and speech problems. A wrap-around retainer is used when extraction is also included in treatment. Wrap-around is utilised as an alternative when the traditional Hawley is liable for occlusal interference. Distortion can happen due to elongated wire and can be further weakened by mishandling the appliances during removal or fitting. Hence, the thumb or forefinger should be used to remove the appliance from the palatal cyclic; on the other hand, the tongue can be used by experienced patients to remove the appliance (Lorenzoni et al., 2019).

In general, retainers are not so appealing in terms of looks. However, vacuum-formed retainers (VFR) are quite appealing since they are invisible retainers of thermoplastic materials such as polypropylene and polyethylene polymers. Polyethylene polymers are more transparent, and they allow bonding to acrylic.

Another type of retainer is a fixed retainer, which is considered necessary for permanent retention. Fixed retainers are made of metal wires of nickel, titanium, or stainless steel. The patient can tolerate well-fixed retainers compared to removable retainers. Fixed retainers offer long-term retention when compared to removable retainers. However, some disadvantages of using fixed retainers are a very high fracture risk and unwanted tooth movement due to wire distortion. Another type of fixed retainer integrates with fibreglass fibres. However, when both types of retainers are compared, it has been revealed that metal wire retainers promote less plaque accumulation and have low failure rates (Mummolo et al., 2022).

Apart from using retainers, there are some scientific ways to reduce relapse, such as bisphosphonates (Fiorillo et al., 2022). Bisphosphonates are drugs that are very effective in reducing the incidences of fractures as they increase the density of the bone, which is the reason why bisphosphonates are used for several metabolic and oncological conditions and used for treating affected bone tissues that cause various bone-related problems such as osteoporosis, multiple myeloma. They are deposited in bone tissue since they possess the characteristics of binding calcium.

Bisphosphonates inhibit bone resorptive function by osteoclasts and help in reducing orthodontic relapse. Bisphosphonate risedronate hydrogel plays a vital role in the process of bone formation (Utari et al., 2022). It carries significant potential for improving the stability of the tooth following orthodontic movement. Plus, it prevents the impending relapse since the intracellular application of bisphosphonate risedronate affected the ratio of osteoclast/osteoblast and alkaline phosphatase (ALP) levels were increased.

Researchers also employ relaxin as another scientific method. Relaxin was first discovered by Dr. Frederick Hisaw in 1926 (Martins et al., 2020). It is an organically developing hormone in the human body. The effect of relaxin indicates that variation of PDL can be created, which in turn can explicitly impact the orthodontic movement of the tooth. Both hard tissue and soft tissue respond to orthodontic tooth movement; however, soft tissue is the one that responds first.; The tooth initially lacks direct contact with soft tissues, but through the periodontal ligament (PDL), it can establish direct contact. This is made possible by relaxin's ability to remodel the soft tissues;

hence, relaxin use in orthodontic movement is being considered. The gingival response is utilised to proliferate the formation of collagen in order to resist the movement. Relaxin is well known for its effects on remodelling soft tissue and is clinically helpful in speeding tooth movement and preventing relapse (Breining et al., 2005). Moreover, this gingival memory is vital in orthodontic relapse after treatment (Yuhan et al., 2020).

Relaxin is used to remodel the soft tissue rather than bone. However, the outcome of the randomised clinical trial by Virdi et al. (2021) by employing recombinant human relaxin demonstrated no substantial difference regarding tooth movement and tooth relapse between the relaxing and placebo control groups. In various in vivo and in vitro studies, Anti-fibrotic properties of connective tissue have been evaluated using relaxin (Virdi et al., 2021). Author Sidhu (2019) experimented with the placebo group and relaxin, and from the experiment, it was identified that there was no significant difference with regard to orthodontic relapse.

Relaxin increases the expression of MMP-1 and MMP-8 in the PDL. Therefore, the collegen's metabolism in the PDL is stimulated by relaxin, which helps prevent orthodontic relapse following the orthodontic treatment (Trehan et al., 2022). In PDL, relaxin disorganises the periodontal fibres, decreases their mechanical strength, and loosens their arrangement.

Raloxifene is a selective oestrogen receptor modulator (SERM) that reduces relapse when administered during retention and the relapse period (Azami et al., 2020). Raloxifene has the advantage of inducing the formation of bone concurrently while averting the resorption of bones. The impact of raloxifene on bone tissue is very similar to oestrogen, but along with limited side effects. It is due to osteoblastogenesis. The main active component that is used to reduce the relapse is psoralen. Hence, psoralen can reduce bone resorption (Kaklamanos et al., 2021).

A non-collagenous matrix protein present profusely in bone tissue is known as osteocalcin, also called bone GLA protein. Osteocalcin constitutes approximately 3% of bone protein (Yuhan et al., 2020). Osteocalcin acts as a negative regulator of mineral apposition, and its high-binding strength with calcium (Ca) and hydroxyapatite helps bone formation. Local Osteocalcin was injected in the first molars of rats, and the movement of the tooth was evaluated for ten days. From the results, it was identified that osteocalcin accelerated the movement of the tooth and also the ability to reduce the relapse. Studies have shown that osteocalcin can enhance the activity and differentiation of osteoblasts, leading to increased bone remodelling and faster tooth movement. In addition, osteocalcin can also stimulate the production of specific cytokines (small proteins involved in immune system signalling), which help to modulate inflammation and prevent relapse (Akin & Akbaydogan, 2022).

Vitamin D, parathyroid hormone, and calcitonin (PTH) regulate phosphorus and calcium levels. Receptors of vitamin D are not only established in osteoblasts but are also present in active osteoclasts and osteoclast precursors. Osteoclasts can be increased by dihydroxyl cholecalciferol and intraligamentary injections of vitamin D metabolite, which also helps reduce the relapse effectively (Sidhu, 2019). Since 1,25 dihydroxy vitamin D3 is an active form of vitamin D, it causes calcium reabsorption. 1,25 dihydroxy vitamin D3 leads to bone resorption due to its similar action on bone. Various experiments were conducted on rats, from which it was identified that 1,25 dihydroxy vitamin D3 is more efficient and effective for bone remodelling, automatically reducing the bone's relapse during orthodontic tooth movement (Virdi et al., 2021).

Kawakami and Takano-Yamamoto (2004) evaluated the effect of 1,25-dihydroxy vitamin D3 on alveolar bone formation during tooth movement in rats. Repeated injections of 1,25 dihyrdoxy vitamin D3 in the orthodontically treated animals distinctly stimulated alveolar bone formation on the mesial side at 14 days. In addition, there was a significant increase in mineral appositional rate associated with elevated osteoblast on the tension surface. These findings suggest that local application of 1,25 dihyrdoxy vitamin D3 enhances the reestablishment of supporting tissue, especially the alveolar bone of teeth, after orthodontic treatment (Kawakami & Takano-Yamamoto, 2004).

Statins help in increasing the formation of bone by inhibiting the apoptosis of osteoblasts. Since bone undergoes constant remodelling, statin plays a vital role as it inhibits enzymes involved with tissue degradation, enhances epithelialisation, and helps heal wounds. Some concerns may be raised during orthodontic treatment, which is related to the association of statin therapy with the growth of osteogenesis and suppression of bone resorption. Using statins to prevent osteogenesis demonstrates a viable therapeutic strategy that minimises orthodontic relapse. Statin also possesses several significant and favourable effects regarding oral health, which not only includes orthodontic relapse but also helps in healing wounded tissues, chronic periodontics, fight against anti-cancer (Tahamtan et al., 2020).

Prostaglandins are the hormones that are responsible for the process of inflammation after the orthodontic tooth movement. It is a group of lipids that helps in tissue repair and the infection connected to injury and illness. Mainly, it helps in the bone resorption of the teeth after the treatment process. Resorption of roots and bones, minimising the synthesis of collagen, is stimulated by prostaglandin. Already existing osteoclasts are activated by prostaglandin. Prostaglandin facilitates tooth mobility at lower doses, whereas root resorption transpires at higher quantities (Bamal et al., 2021).

Osteoprotegerin (OPG) is an examined element that can control and reduce relapse without primary systemic results on long bones. OPG is a decoy receptor for the receptor activator of nuclear factor kappa beta. OPG performs as a competitive inhibitor of receptor activator of nuclear factor, obstructing osteoclastogenesis and the following resorption of bone (Lee, 2022).

Nanomaterials hold a unique position in the field of dentistry, particularly in tissue engineering, where they are used to repair bone defects caused by various factors. These materials are broadly categorised into bioactive and bioinert materials based on their interaction with biological tissues.

Bioactive nanomaterials interact with biological tissues to promote healing and regeneration. Examples include hydroxyapatite (HA) nanoparticles, calcium phosphate nanoparticles, and bioactive glass nanoparticles (Kim et al., 2016). Nanofibers such as collagen and gelatin nanofibers are also commonly used (O'Brien, 2011). Additionally, nanocomposites like hydroxyapatite/polymer nanocomposites and collagen/nanohydroxyapatite composites are widely applied in bone regeneration (Rezwan et al., 2006). Carbon nanotubes (CNTs), including single-walled and multi-walled carbon nanotubes, are another important class of bioactive nanomaterials with applications in bone tissue engineering (Mohan et al., 2014).

Bioinert materials, on the other hand, are designed to be non-reactive within the biological environment while supporting tissue engineering applications. Polycaprolactone (PCL), a biodegradable polymer, is frequently used in bone tissue engineering to facilitate cell growth and differentiation (Khan & Tanaka, 2017).

Polyethylene glycol (PEG) is another bioinert material that can be functionalised to create bioactive scaffolds for improved cell interaction (Khan & Tanaka, 2017).

This distinction between bioactive and bioinert materials highlights their diverse roles in advancing dental tissue engineering and bone regeneration (Laurencin & Khan, 2012).

Nanomaterials perform better in repairing the bones' tissues than alloplastic materials (Tanaka et al., 2020). It supports the proliferation of the cells. In vivo, such materials are commonly tested in animal bone defect models to assess their bone regeneration potential. They can be used as fillers to strengthen bone regeneration scaffolds (Wang et al., 2016).

Carbon nanotube (CNT) is a kind of nanomaterial that appears to be cylindrical (Raphey et al., 2019). The graphene sheet is covered as an outer layer with carbon atoms. The appearance of the structure of CNT paves the way for its use in a diverse range of bio-medical stream applications. The graphene is wrapped up, which forms the cylinder in CNT composition. The difference is shown in the multi-wall carbon nanotube (MWCNT) and single-wall carbon nanotube (SWCNT) by the layer of the graphene sheet in the cylinders (Raphey et al., 2019). Researchers are extensively exploring carbon nanotubes (CNTs), particularly multi-walled carbon nanotubes (MWCNTs), for their unique properties in bone regeneration. These nanotubes support bone growth and function by interacting with macromolecules and promoting cell adhesion. Among the various nanomaterials used in bone regeneration, CNTs combined with active biological materials stand out. MWCNTs, in particular, enhance osteogenic differentiation and improve cell adhesion. Their structural features also allow the incorporation of growth factors, further stimulating bone development (Henna et al., 2020).

Several scientific studies confirmed its effectiveness by checking osteoblasts adhesion to such complex, influence on the proliferation of osteoblasts and osteocytes, and MWCNTs' promotion of osseous tissue formation in vivo (Saito et al., 2008). Tanaka et al. (2017) confirmed that MWCNT blocks could serve as filler materials because they are solid, with nano-sized surface irregularities and non-porous interiors, preventing surrounding cells from entering the scaffold. Such a scaffold can have osteoconductive abilities by allowing osteoblasts to proliferate on the MWCNT block surface. Their previous studies about MWCNT also proved that this material can be successfully used as a functional scaffold for bone formation and promote bone tissue regeneration (Shimizu et al., 2012).

CNT has the potential to be used in bone tissue engineering. However, for safe usage in humans, it is necessary to ensure it possesses no toxicity (Almeida et al., 2020). It was scientifically found that CNTs can be a safe, new, and high-performance biomaterial by controlling their type, administration site, and dosage (Aoki & Saito, 2020). Liu et al. (2015) suggested that MWCNTs-COOH might be safer for in vivo application. Concerning the biocompatibility and toxicity of carbon nanostructures, the results of the previous study showed that they are suitable for biological applications (Henna et al., 2020).

The CNT's polymeric composites make it suitable for tissue engineering. The main reason for using the CNT in tissue regeneration is its physical strength, electrical properties, and thermal expansion, making it a supplement that improves the polymeric composite features (Aoki & Saito, 2020). The CNT is used to strengthen the bioactivity process and enrich the mechanical properties. Moreover, in developing the cell culture and its proliferation in osteoblast fibroblast, the CNT has given a preferable outcome supporting repairing the bones' tissues (Aoki & Saito, 2020).

Two things are present in platelet-rich plasma (PRP). One is plasma, and another one is platelets. Platelets are present in the blood, which is disc-shaped and large. Platelets possess inherent wound-healing capabilities. It aids in halting haemorrhage and decelerating the development of thrombi (Kendall, 2020). The PRP injection is prepared by taking the blood sample from the patients into the centrifuge device, and the concentrated sample of the plasma is mixed to form the PRP. PRP triggers the production of the cells, acts as a stimulus in the regeneration process of the tissues, and heals the wounds in the specific area. It mainly triggers the development of bone regeneration because of the growth factors present in it (Kendall, 2020).

PRP is widely used in tissue engineering and cell-based therapy with the help of autologous plasma and also with concentrated platelets. Almost 300 active molecules are contained in platelets released upon activation from platelet alpha, which helps regulate the tissue regeneration procedure. In regenerative medicine, the bioactive material is crucial, including healing wounds, bone remodelling, and nerve regeneration (Xu et al., 2020). Because of the bioactive molecules, the PRP is utilised as a regenerative medicine in diverse applications, such as remodelling of the bones, which is very helpful in dentistry, such as orthodontic treatments.

PRP is used in dentistry when there are issues like periodontitis, bone grafts, extraction of a tooth and other. PRP injections are used only when patients undergo complicated invasive surgical procedures or severe oral diseases (Jaafer et al., 2020).

PRP is used in the dental field since it allows the dentist to use the body's organic way of healing, which is much quicker and more efficient. Studies show that patients benefit significantly because of PRP, which is 6 times faster than the conventional way of healing wounds.

The migration of the PDL cells takes place in the periodontal regeneration. In that process, wound cells need to be maintained for the migration process of the cells. The growth factors present in the PRP will provide the space for the migration process of cells. In periodontics, it is used for plastic surgery in the periodontium and maxillofacial surgery, which involves tooth extraction, Surgeries in the soft tissues and the bone tissue, and tooth implants (Xu et al., 2020).

There is scientific evidence that PRP is promising and can be used in oral, dental, and maxillofacial procedures. It helps in the proliferation of the osteoblast cells in the tissues of the tooth. The growth factors that are present support repairing the tissues of the tooth and regeneration of the bones of the tooth, stimulate the preosteoclast cells for the process of mitosis and also hike the numbers of chemotaxis, which will initiate the osteoblast cells for the regeneration and mineralisation of the cells and healing the tissues of the teeth. These present factors help repair the tissues after the orthodontic treatments. PRP is very successful in the dental practices. Because of the growth factors, PRP is mostly recommended to be used to heal the tissues and the osteoblast cells after the orthodontic treatments, increasing the density of the bones and reducing pain (Zhang et al., 2021).

Several factors can contribute to relapse, e.g., lack of retention, genetics, age, incomplete treatment, habits, poor oral hygiene, and natural ageing. One of the primary causes of relapse is inadequate or inconsistent use of retainers (Thilander, 2000).

Orthodontic treatment at a younger age may have a higher likelihood of relapse due to ongoing skeletal growth and dental changes as they develop. If the orthodontic treatment plan is not fully completed, such as not addressing all underlying issues or not achieving ideal alignment, it can increase the chance of relapse (Melrose & Millett, 1998). Certain habits, such as thumb or finger sucking, tongue thrusting, or prolonged use of pacifiers, can exert pressure on teeth, leading to relapse. As with age, the tissues supporting the teeth tend to undergo changes, which can contribute to relapse over time. MWCNT incorporated into materials used for orthodontic retainers can increase their mechanical properties, such as stiffness and elasticity (Zhylich & Suri, 2011). This enhanced material strength potentially provides better support and stability for the teeth, thus reducing the risk of relapse. PRP contains growth factors and proteins that can aid in tissue regeneration and healing (Liu et al., 2022). PRP is applied to the orthodontic site after tooth movement in order to promote faster and more efficient bone remodelling by accelerating the healing process and reducing the likelihood of relapse.

1.2 Problem Statement

In recent years, orthodontic treatment has become increasingly necessary in advanced societies. However, orthodontic relapse is a significant concern that affects patient expectations (Littlewood et al., 2017). Several studies have highlighted the downsides of the existing retainers, e.g., fixed lingual retainer (Kartal & Kaya, 2019), Hawley retainer (Alassiry, 2019), Essix and Vivera retainer (Zafeiriadis et al., 2018). Therefore, an innovative approach is needed to reduce orthodontic relapse.

Relapse can be reduced in several ways (Hu et al., 2022); however, minimal research has been inspected the implementation of nanotechnology for reducing orthodontic relapse (Al-Dboush & El-Bialy, 2021; Gauba et al., 2022), especially by combining biological materials and chemical materials of MWCNT and PRP for orthodontic relapse. This approach to reducing relapse has not been examined at the moment.

Histological analyses are essential to assess the impact of MWCNTs and PRP on the activation of osteoblasts and the inhibition of osteoclasts. Although previous studies provided basic histological insights into the use of MWCNTs, their application was limited to general used in biomedical field (Zare et al., 2021). Another study identified the histological modifications followed by the cytotoxicity of using carbon nanotubes for dogs' bone grafts. It concluded that MWCNT was non-toxic when used at a safety level (Mitra et al., 2022). However, adverse histological changes of the functionalised MWCNT combined with PRP for the rabbit model have not been revealed in any study. Hence, a substantial gap exists between the knowledge and literature on employing the functionalised MWCNT to reduce orthodontic relapse through their mechanical properties, such as stiffness and elasticity.

The use of CNTs in bone remodelling has shown promising results, suggesting that it has the potential to bring stability to orthodontic treatment (Castro-Rojas et al., 2021). Therefore, this study seeks to investigate the multi-walled carbon nanotubes (MWCNTs) and/or platelet-rich plasma (PRP) in minimising orthodontic relapse.

1.3 Research Questions (RQ)

RQ1. What are the effects of MWCNTs, PRP, and a combination of both materials on the clinical orthodontic tooth relapse in rabbits?

RQ2. What are the effects of MWCNTs, PRP, and a combination of both materials on histological analysis of mandibular dentoalveolar structure in rabbits?

1.4 Objectives of the Study

1.4.1 General Objective

This study investigates the effect of MWCNTs, PRP, and the combination of both materials on orthodontic tooth relapse following orthodontic tooth movement.

1.4.2 Specific Objective (RO)

RO1. To compare the amount of orthodontic tooth relapse in rabbits intervened with MWCNTs, PRP, and a combination of both materials by using an Electrical Digital Vernier (EDV).

RO2. To compare the amount of orthodontic tooth relapse in rabbits intervened with MWCNTs, PRP, and a combination of both materials by using a Cone Beam Computed Tomography (CBCT).

RO3. To compare the amount of bone density in rabbits intervened with MWCNTs, PRP, and a combination of both materials by using a Cone Beam Computed Tomography (CBCT).

RO4. To evaluate the qualitative histological analysis of the mandibular dentoalveolar structure in rabbits intervened with MWCNTs, PRP, and a combination of both materials using Haematoxylin and Eosin (H & E).

RO5. To evaluate the quantitative histological (histomorphometric) analysis of the mandibular dentoalveolar structure in rabbits intervened with MWCNTs, PRP, and a combination of both materials using Haematoxylin and Eosin (H & E).

1.5 Research Hypothesis

Multiple studies have highlighted the possible benefits of MWCNTs and PRP in supporting bone formation and reducing relapse, respectively. Building on existing research, it is proposed that the combined application of MWCNTs and PRP may have a more pronounced effect on reducing orthodontic tooth relapse and promoting bone formation, potentially resulting in unique histological changes when tested in rabbits.

Hypothesis I : There is no difference in the amount of orthodontic tooth relapse in rabbits that are intervened with MWCNTs, PRP, and a combination of both materials by using an Electrical Digital Vernier (EDV).

Hypothesis II : There is no difference in the amount of orthodontic tooth relapse in rabbits that are intervened with MWCNTs, PRP, and a combination of both materials by using a Cone Beam Computed Tomography (CBCT).

Hypothesis III : There is no difference in the amount of bone density in rabbits that are intervened with MWCNTs, PRP, and a combination of both materials by using a Cone Beam Computed Tomography (CBCT).

Hypothesis IV : There is no difference in the qualitative histological analysis of the mandibular dentoalveolar structure in rabbits that are intervention with MWCNTs, PRP, and a combination of both materials using Haematoxylin and Eosin (H & E).

Hypothesis V : There is no difference in the quantitative histological (histomorphometric) analysis of the mandibular dentoalveolar structure in rabbits that are intervention with MWCNTs, PRP, and a combination of both materials using Haematoxylin and Eosin (H & E).

CHAPTER 2

LITERATURE REVIEW

2.1 Malocclusion: Prevalence and Aetiology

According to the World Health Organisation, orthodontic malocclusion is considered one of the most common oral complaints after tooth decay and periodontitis (Alhammadi et al., 2018). It has been reported that there is no specific etiology responsible for malocclusion. Malocclusion is related to multiple predisposing factors, including hereditary, environmental, or a combination (Alhammadi et al., 2018).

In 2021, Cenzato et al. reported that genetic effects are principally exhibited during growth and development, resulting in an increased risk of developing malalignment. However, specific acquired elements could promote these hereditary traits, including malfunctioning habits (Cenzato et al., 2021). Thumb sucking is one of the harmful oral habits that greatly encroaches on palatal development. Subsequently, it triggers the creation of severe malaligned situations such as open bite anteriorly or crossbite posteriorly (Abate et al., 2020).

Oral respiration is a noteworthy oral habit that demands careful consideration owing to its substantial influence on the development of malocclusion. This habit typically arises from various physiological factors predisposing nasal passage obstruction, compelling individuals to resort to alternative breathing pathways, as observed in conditions like adenoids, allergic rhinitis, or chronic sinusitis. Prolonged oral breathing is likely to contribute to malalignment of teeth and adversely affect skeletal bony development. Manifestations may include anterior open bite, protrusive mandibular growth, and a constricted palate (Cenzato et al., 2021; Muñoz & Orta, 2014). These factors and others are suggested to play an essential role in the higher preponderance of malocclusion nowadays compared to the past (Alqahtan et al., 2020).

2.2 Importance of Orthodontics

Consequently, requests for orthodontic treatment have remarkably increased throughout the world. The escalating demand for orthodontic intervention in contemporary society primarily stems from the adverse effects of dental irregularities and occlusal abnormalities on individual aesthetics. Consequently, orthodontic patients often grapple with psychological repercussions linked to their appearance. Moreover, malocclusion further compromises oral health and essential functions, significantly impacting overall human well-being (Hamid et al., 2022).

2.2.1 Psychological Adverse Effects

Undoubtedly, facial appearance is greatly impacting individual confidence and psychological balance. Literature has indicated that extensive malocclusion most probably impedes social interactions. Regularly arranged teeth and an appealing smile improve personal psychological status and social relationships in general (Meyer-Marcotty et al., 2010). That is mainly observed in children's and adolescents' performance in schools and in females whose aesthetics are considered of optimum value (Bresnahan et al., 2010).

2.2.2 Oral Function Hindrance

Food mastication is negatively affected by extensive malocclusion. That most likely appears as an ineffective chewing procedure, resulting in the patient avoiding chewing difficulties by refraining from eating certain foods or swallowing food without sufficient chomping. This could initiate a cascade of gastrointestinal disorders or disturbances in general nutrition. Furthermore, the phonetic function may be partially compromised due to severely malaligned teeth, despite the patient's efforts to perform abnormal adaptation movements of the lips and tongue to achieve harmonious speech.

Deleterious reactions of temporomandibular joint TMJ to malocclusion have been widely investigated. It is widely acknowledged that minor disturbances in the occlusal apparatus may play a role in initiating or contributing to dysfunctional habits like clenching or bruxism. The manifestations of temporomandibular disturbances, as seen in TMD, such as pain and clicking, may consequently ensue. While a definitive causative relationship between malocclusion and TMJ diseases has not been conclusively established, orthodontic treatment has been suggested as a potential adjunct to alleviate TMD symptoms (Macfarlane et al., 2009).

2.2.3 Oral Health Impediment

Although there is no obvious scientific evidence regarding the existence of a direct connection between malocclusion and the development of oral diseases (Jonsson et al., 2010), it has been frequently stated that occlusal abnormalities could indirectly superimpose dental caries or periodontitis by hampering the oral hygiene measures. Furthermore, research indicates that approximately one-third of children experience Class II malocclusion, characterised by the protrusion of upper central incisors, putting them at risk of traumatic fractures in these specific teeth. In cases of severe overbite, there is a heightened risk of lower anterior teeth making contact with the palate, potentially causing soft tissue injuries. Additionally, extensive tissue destruction may impact upper incisors, increasing the likelihood of their loss. Therefore, orthodontic treatment has been confirmed mandatory to promote quality of life (Proffit et al., 2019).

2.3 Orthodontic Treatment Appliances

Following diagnosis of the orthodontic demand, treatment planning is designed in a specific method that achieves the required goal, considering the patient's concerns and expectations simultaneously. Throughout the past few decades, various Orthodontic appliances have been gradually introduced to fulfil the expanding eligibility criteria. Numerous factors are merging to create a suitable appliance, including.

i) The functional capability of the orthodontic device to achieve optimum results in the least time frame.

ii) The aesthetic values of the device are also quite significant to ensure patient satisfaction.

iii) Financial concerns must also be considered (Alansari et al., 2019).

Therefore, different orthodontic appliances have been invented and applied for diverse scenarios. The principal two types of orthodontic devices are fixed versus removable appliances. Since Dewel et al. (1971), literature has widely investigated the strengths and weaknesses of both categories. However, it has been evidenced that most successful outcomes of fixed appliances outweigh those of removable correspondents (O’Brien et al., 1993; Richmond et al., 1993). Subsequently, fixed appliances have emerged as the primary modality for orthodontic treatment, especially in cases of severe malalignment.

2.4 Fixed Orthodontic Appliances

Angle system, designed in 1987, has been the backbone of the majority of the fixed appliances known in the present time, including:

i) E arch appliance.

ii) Pin and tube appliance.

iii) Ribbon arch device.

iv) Edgewise appliances.

In addition, fixed appliances have been preferred over removable ones due to several advantages they generally exhibit, such as the following:

i) Fixed devices display tooth motions, such as tipping, translation, displacement, rotation, and intrusive and extrusive movements.

ii) They could be utilised for the fixation of different malocclusion situations.

iii) They could be adjusted so a combination of tooth movements is developed simultaneously.

iv) Fixed appliances exhibit a superior range of anchorage.

v) Accurate and detail-oriented teeth motion can be achieved by fixed Orthodontics (Alam, 2012).

On the other hand, certain drawbacks of fixed devices have been stated, including:

i) The hardship of applying oral hygiene measures could result in food impaction and aggregation of plaque and calculus.

ii) Advanced training is mandatory to install fixed appliances correctly to avoid undesirable outcomes or disastrous complications.

iii) In urgent situations such as broken parts, fixed appliances require a specialist to detach the device that is not easily accessible.

iv) Fixed orthodontics presents a financial burden to the patient compared to removable appliances (Alam, 2012).

2.5 Mechanism of Orthodontic Movement

Diverse implementations for fixed orthodontic appliances have been applied for managing many malocclusion situations, namely open bite, crossbite, separated teeth, crooking, bulged teeth, and various skeletal bone defects (Jacob et al., 2017).

The fundamental principal underpinning OTM is predicated on the thesis that the application of sustained pressure forces on the tooth structure instigates intricate cascades within the surrounding periodontium. These cascades, occurring over an extended duration, ultimately facilitate the process of orthodontic tooth movement (Prameswari et al., 2020). The time frame required to accomplish the desired results is an essential concern for the patient. The duration of the orthodontic treatment differs considerably according to the nature of malalignment and the treatment planning being applied. In addition, other factors influence the treatment time, such as practitioner qualifications and patient cooperation. A wide range of time frames has been introduced in the literature (Moresca, 2018). In 2016, Tsichlaki et al., in their Systematic review, estimated that the treatment time with fixed appliances ranges between 14 to 33 months without investigating the treatment results (Tsichlaki et al., 2016).

Moreover, the average time of orthodontic treatment, estimated by the American Board of Orthodontics (ABO), is approximately 25 months (Aljehani & Baeshen, 2018). The primary concern for orthodontic patients often revolves around the reduction of treatment time. Consequently, significant efforts have been dedicated to comprehending the intricate mechanisms underlying orthodontic movement. This understanding serves as a foundational point for endeavours aimed at minimising the duration of orthodontic treatment (Moresca, 2018).

2.5.1 Brustone Phases of Orthodontic Tooth Movement

In 1962, Burstone introduced a detailed description of the mechanism of orthodontic tooth movement by dividing the process into 3 phases (Burstone, 1962):

i) **Initial phase:** When the tooth is subjected to external force, its first reaction rapidly moves in the scope of the periodontium and bony socket. This motion generates pressure on one side of the PDL and tension on the corresponding side. This reaction provokes inflammatory responses, leading to vasodilation chemotaxis of the inflammatory cells and bone cells. This phase usually occurs within 24 -48 hours (Asiry, 2018).

ii) **Lag phase:** As a result of the inflammatory cascade induced in the compacted periodontal tissues, hyaline degeneration occurs in the pressure side for bone and periodontium, leaving necrotic tissues filling the space that is supposed to be utilised for tooth movement (Kashyap, 2016). Hence, orthodontic tooth movement significantly diminishes during this phase, wherein scavenger cells, such as macrophages and osteoclasts, actively remove or engulf the hyalinised tissues. The lag