Needle-free Liquid Jet Injection for Dental Anesthesia

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Nov 2021

Montreal, QC, Canada

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy in Oral Health Sciences

Abstract

Needle-Free Liquid Jet Injection (NFLJI) systems can deliver therapeutic fluid into the body without the need for needle injection. These systems work by creating a thin (usually 76-360 μm in diameter) and high-velocity (typically >100m s-1) liquid jet that penetrates through the skin. The use of NFLJI can eliminate the risks and complications of needle injection, i.e., needle phobia. However, the NFLJI systems are rarely used in dental practice, mainly due to inconsistent outcomes and unpredictable complications, such as bleeding and discomfort, unpleasant taste, and unpredictable pain. This dissertation aimed to develop and optimize techniques for needle-free dental anesthesia, namely infiltration and mental incisive nerve block (MINB). To achieve the aims of this thesis, NFLJI was first investigated *in vitro* and on cadavers. Its clinical safety and feasibility were evaluated in pilot randomized controlled trials (RCT).

Infiltration anesthesia delivers anesthetics to the superficial nerve ends at the dentoalveolar regions, which have a thin layer of mucosa supported by rigid bone. Our *in vitro* experiments showed that perpendicular NFLJI created significant fluid regurgitation when injected into soft tissue supported by hard tissue, which was also confirmed in cadavers. Moreover, Clinical trials revealed that perpendicular NFLJIs induced a high risk of bleeding (83.3%) and laceration (83.3%). This issue could be avoided by modifying the injection angle. Oblique NFLJIs induced significantly less regurgitation *in vitro* than perpendicular ones. It also showed a low risk of bleeding (33.3 %) and laceration (16.7%) *in vivo*. The preliminary success rates of oblique NFLJIs and needle injections were both 83.3%.

Unlike infiltration anesthesia, dental nerve blocks require deeper penetration depth into relatively thicker tissue to target the main nerve branches. *In vitro* experiment revealed that the

NFLJI penetration depth was directly proportional to the supply pressure and drug volume, and it can achieve sufficient penetration depth for nerve blocks. However, increasing NFLJI supply pressure can also increase the maximum force, total work, jet velocity, jet impingement pressure, and jet penetration pressure. High-pressure NFLJIs (620 kPa) created maximum force and total work significantly greater than needle injections *in vivo*, and they resulted in a high prevalence of discomfort (60%) and paresthesia (20%) in pilot RCT. The pilot RCT was stopped due to the nerve paresthesia caused by high-pressure NFLJI. This issue was minimized by employing a low-pressure NFLJI (413 kPa). Low-pressure NFLJIs created a maximum force and total work similar to those of needle injections. Besides, they also created significantly lower jet impingement pressure and jet penetration pressure than high-speed NFLJIs, indicating a lower risk of nerve damage. Pilot RCT revealed that low-pressure NFLJIs were less likely to cause any complications (0%). The preliminary success rates of MINB using NFLJIs and needles were 83.3 and 87.5%, respectively, on cadavers; and 60% and 70%, respectively, in clinical trials.

In conclusion, by optimizing the injection angle and pressure, NFLJI could be successfully used in regular dental anesthesia procedures such as infiltration and MINB. Oblique injection angles can minimize the complications such as drug regurgitation and tissue laceration when injecting at the dentoalveolar region. Low-pressure NFLJI can create sufficient penetration depth for MINB while maintaining relatively low penetration pressure to avoid nerve and tissue damage. The pilot RCTs confirmed the feasibility of conducting a non-inferiority RCT to further evaluate the findings in this thesis.

Résumé

Les systèmes d'Injection Sans Aiguille par Jet de Liquide (ISAJL) peuvent administrer un fluide thérapeutique dans le corps sans avoir recours à une aiguille. Ces systèmes fonctionnent en créant un fin jet de liquide (généralement de 76 à 360 µm de diamètre), expulsé à grande vitesse (généralement > 100 m s-1) pénétrant ainsi à travers la peau. L'utilisation de ISAJL peut éliminer les risques et les complications causées par les injections traditionnelles, notamment la phobie des aiguilles. Cependant, les systèmes d'ISAJL sont rares dans la pratique dentaire, principalement en raison de résultats incohérents et de complications telles que des saignements, de l'inconfort, un goût désagréable ou une douleur imprévisible. La recherche présentée ici visait ainsi à développer et optimiser les techniques d'anesthésie dentaire sans aiguille, comme l'infiltration et le blocage nerveux incisif mental (BNIM). Pour atteindre les objectifs de cette thèse, l'ISAJL a d'abord été étudié in vitro et sur des cadavres, puis sa sécurité clinique et sa faisabilité ont été évaluées dans des essais contrôlés aléatoires (ECA) pilotes.

L'anesthésie par infiltration délivre des produits anesthésiques aux extrémités nerveuses superficielles des régions dentoalvéolaires, qui ont une fine couche de muqueuse soutenue par un os rigide. Nos expériences in vitro ont montré que l'ISAJL perpendiculaire créait une régurgitation de fluide significative lors d'une injection dans des tissus mous soutenus par des tissus durs. Ces résultats ont été confirmés sur des cadavres. De plus, des essais cliniques ont révélé que les ISAJL perpendiculaires induisaient un risque élevé de saignement (83 %) et de lacération (83 %). Ce problème pourrait être évité en modifiant l'angle d'injection. En effet, les ISAJL obliques induisent significativement moins de régurgitation in vitro que les perpendiculaires puis ont réduit la prévalence d'hémorragie (33 %) et de lacération (16 %) in

vivo. Dans le cadre de notre ECA, nous avons obtenu un taux de réussite préliminaire de 83% pour les ISAJL obliques ainsi que pour les injections à l'aiguille.

Contrairement à l'anesthésie par infiltration, les blocages nerveux dentaires nécessitent une profondeur de pénétration plus profonde dans des tissus épais pour atteindre les principales branches nerveuses. Une expérience in vitro a révélé que la profondeur de pénétration du ISAJL était directement proportionnelle à la pression d'injection et au volume de médicament et qu'elle peut atteindre une profondeur de pénétration suffisante pour les procéder au blocage nerveux. Cependant, l'augmentation de la pression de l'ISAJL peut également causer une augmentation de la force maximale, du travail total opéré par le jet, de la vitesse du jet, de la pression d'impact du jet et de la pression de pénétration du jet. Les ISAJL à haute pression (620 kPa) ont créé une force maximale et un travail total significativement supérieurs aux injections traditionnelles in vivo et ils ont entraîné une prévalence élevée d'inconfort (60 %) et de paresthésie (20 %) dans l'ECA pilote. L'ECA pilote a été arrêté en raison de la paresthésie nerveuse causée par le ISAJL à haute pression. Ce problème a pu être minimisé en utilisant un ISAJL à basse pression (413 kPa). Les ISAJL à basse pression ont créé une force maximale et un travail total similaires à ceux des injections à l'aiguille. En outre, elles ont également créé des pressions d'impact et de pénétration nettement inférieures à celles des ISAJL à grande vitesse, ce qui cause un risque plus faible de lésions nerveuses. L'ECA pilote a révélé que les ISAJL à basse pression étaient moins susceptibles de causer des complications (0 %). Les taux de réussite préliminaires du BNIM utilisant des ISAJL et des aiguilles étaient de 83% et 88%, respectivement, sur des cadavres; et 60 % et 70 %, respectivement, dans les essais cliniques.

En conclusion, en optimisant l'angle et la pression d'injection, l'ISAJL pourrait être utilisée dans les procédures d'anesthésie dentaire les plus communes telles que l'infiltration et le

BNIM. Des angles d'injection obliques peuvent minimiser les complications telles que la régurgitation du médicament et la lacération des tissus lors d'injections dans la région dentoalvéolaire. L'ISAJL à basse pression peut créer une profondeur de pénétration suffisante pour le BNIM tout en maintenant une pression de pénétration relativement faible pour éviter les lésions nerveuses et tissulaires. Les ECA pilotes effectués ont confirmé la faisabilité de mener un ECA de non-infériorité pour évaluer davantage les résultats obtenus dans le cadre de la présente recherche.

Acknowledgment

Pursuing a Ph.D. degree is a life-changing experience, bringing failures, challenges, courage, wisdom, and growth to my life. I would not be able to go through these challenges without the support and guidance from many people.

First and foremost, I would thank my supervisors, Profs. Faleh Tamimi and Luc Mongeau, who gave me invaluable support, guidance, and encouragement. The achievements during my studies would not have been possible without their feedback, advice, and vast knowledge. They also provided me honest career advice, support me to take numerous travel opportunities to learn and collaborate with international researchers across the world. My gratitude is also extended to my advisory committee member and collaborator. I won't forget all the cadaver experiments Dr. Geoffroy Noel, and I worked on together. And those clinical trial sessions Dr. Zovinar der Khatchadourian worked with us. I must also thank Dr. Elham Emami and Dr. Anna Velly for designing the clinical trial and data analysis and providing sincere career advice. Many thanks to Dr. Laura Stone, Dr. Monzur Murshed, Dr. Zahi Brandon, and Dr. Elise Verron for guiding me in the early stage of my Ph.D. Thank you all for positively influencing my life.

I had the great pleasure of working with all my co-authors; thank you all for contributing to this dissertation. Special thanks to my colleagues from Dr. Tamimi's and Mongeau's Lab: Doaa Taqi, Faez Al-Hamed, Mohammad Abu-Samak, Anna Heley, Zixin He, Guangyu Bao for their endless help and support. I also enjoyed working with and learning many things from Emily Buck, Miyako Suzuki, William Lepry, and Zhenwei Ma. I gratefully acknowledge the technical assistance from Mr. Karim Menassa and his team from Medical International Technology Inc; I'd like to recognize the guidance I received from Haider Al-Waeli, Ahmed Al-Subaie, Alaa

Mansour for teaching me animal surgeries and their career advice. I sincerely appreciate the friendships we built together and the mental support I got from you. I had great memories with all of you, and I wish you all the best in every aspect of your life.

Finally, I'd like to thank the numerous funding source throughout my Ph.D.: the Clifford C.F.Wong Fellowship, the Ph.D. scholarship of Chinese Scholarship Council, Natural Sciences and Engineering Research Council of Canada (NSERC) engage research grant, the Réseau de recherche en santé buccodentaire et osseuse (RSBO) research fellowship and travel award, the alpha omega foundation research grant, the Graduate Research Excellence and Travel Award (GREAT).

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Glossary of Abbreviations and Symbols

NFLJI	Needle-Free Liquid Jet Injection
RCT	Randomized Controlled Trial
MINB	Mental Incisive Nerve Block

Contribution to Original Knowledge

This thesis describes an innovative research methodology to translate knowledge of needle-free liquid jet fluid dynamics in the oral cavity from laboratory to clinical practice as optimized dental infiltration and nerve block anesthesia techniques. A wide range of factors can impact the NFLJI outcomes, the properties of soft tissue and injected fluid, and the parameters of NFLJI. The latter is the only modifiable factor, therefore of great importance. This thesis showed how NFLJI parameters, namely injection angle, supply pressure, and volume, change the outcomes, such as jet penetration, dispersion, regurgitation, and potential tissue damage. This study set the basis for translating NFLJI from bench to dental chair.

This thesis contains three manuscripts: one review and two original research manuscripts. The review paper in chapter 3 was published in a peer-review journal, "Drug Discovery Today." This paper summarized the delivery system for local anesthetics in bone surgery, updated the literature regarding the safety and efficacy of this anesthetics.

Chapter 5 highlighted the importance of jet injection angle for the NFLJI infiltration anesthesia technique. This study demonstrated the unique fluid dynamics of high-speed liquid jets in the dentoalveolar region, identified the reason for inconsistent efficacy and unpredictable complications in previous studies, proposed a solution to mitigate the problem, evaluated the safety and feasibility of the new solution. This manuscript was published in a peer-review journal, "International Journal of Pharmaceutics."

Chapter 6 highlights how supply pressure influences the NFLJI penetration and causing potential tissue damage. This work investigated the relationship between NFLJI penetration depth and its parameters, namely supply pressure and volume, generated a predictive model. The in vitro study analyzed the force-time and depth-time history of NFLJI. This in vitro study

explained why high-pressure NFLJI could cause tissue damage and how low-pressure NFLJI can reduce this risk. The safety and feasibility of NFLJI for mental incisive nerve blocks were validated by the pilot clinical trials. This manuscript was submitted to the "International Journal of Pharmaceutics" and is under review.

Contribution of Authors

Chapter 3 is a review paper published in Drug Discovery Today, with the title "Delivery systems of local anesthetics in bone surgery:are they efficient and safe?".

Contribution of authors: Q. Gao, M. Dupleichs, and E. Verron contributed to the original draft preparation and editing, Z. Badran, P. Janvier, J.M. Bouler, O.Gauthier, F. Tamimi, and E. Verron contributed in the review and editing.

Chapter 5 is an orginal research paper published in the International Journal of Pharmaceutics with the title "Needle-free injection: dental infiltration anesthesia".

Contribution of authors: Q. GAO contributed to the conception, study design, data acquisition, and interpretation of the study, drafted and revised the manuscript. D. TAQI, M. ABUSAMAK, K. MENASSA, Z. He, A. HENLEY, S. Groen, and R. TAYHE contributed to the experimental design, data acquisition, and critically revised the manuscript. G. NOEL and Z. D. KHATCHADOURIAN, A. VELLY, E. ELHAM, L. MONGEAU and F. TAMIMI contributed variously to the conception, design, data interpretation, and revision of the study.

Chapter 6 is also an orginal research paper that was submitted to the International Journal of Pharmaceutics with the title "Needle-free injection: mental incisive nerve block".

Contribution of authors: Q. GAO contributed to the conceptualization, methodology, validation, formal analysis, investigation, data curation, writing of original draft and editing, visualization, and project administration. D. TAQI, M. ABUSAMAK, K. MENASSA, Z. He, A.

HENLEY, S. Groen, and R. TAYHE contributed to the research validation, investigation, data curation, analysis, review and editing of the manuscript. G. NOEL and Z. D. KHATCHADOURIAN, A. VELLY, E. ELHAM, L. MONGEAU, and F. TAMIMI contributed variously to the conceptualization, methodology, investigation, resources, review, and editing of the manuscript. L. MONGEAU and F. TAMIMI also contributed to the Funding acquisition.

All authors gave their final approval and agreed to be accountable for all aspects of the work. The authors declare that there is no conflict of interest associated with this publication. There has been no significant financial support for this work that could have influenced its outcome.

Chapter 1: Introduction

1. Thesis outline

This thesis is prepared in a manuscript-based format according to the McGill University Thesis preparation guidelines, and it is formed by eight chapters. Chapter one includes the general introduction, research rationale, hypothesis, and objectives of the work. Chapter two introduces a literature review of the mechanism of pain and needle phobia for drug administration and the needle-free liquid jet injection system. Chapter three is a published manuscript reviews the current delivery system of local anesthesia for bone surgeries, prepared by the candidate as the co-first author. Chapter four presents the main characterization techniques and analysis methods employed for this work. Chapter five and six include two original research manuscripts prepared by the candidate as the first author. Chapter seven summarizes the findings and general conclusions. Chapter eight explains the limitations of this work and future directions. Chapter nine contains the list of references cited in this thesis, and chapter ten contains the appendix listed the article published by the candidate as first author or co-author during her Ph.D. Studies. Chapter eleven listed the research proposal and questionnaires for clinical trials.

2. Thesis research rationale, hypothesis, and objectives.

The Needle-Free Liquid Jet Injection (NFLJI) systems are powered by gas, laser, or spring pressure to create a thin (usually 76-360 μm in diameter) and a high-velocity (typically >100m s-1) liquid jet, which delivers therapeutic fluid across the skin into the subcutaneous or intramuscular region (*1*). The use of NFLJI eliminates risk or problems from needle injections, such as avoidance behavior due to needle phobia and injection pain(*2-5*), needle fracture (*6*), disease transmission due to needle reuse, and cost of needle disposal (*1*).

Needle-free liquid jet injection (NFLJI) systems have been used in dermatology (*7*) as well as for the delivery of vaccines (*8, 9*), insulin (*10*), and growth hormones (*11*).

Dental anesthesia works by depositing local anesthetics at the region of treatment (infiltration) or at the main nerve branch that controls the pain feeling of the downstream operation region (nerve block); hence the anesthetics can block the pain signal transfer by blocking the sodium channel of the nerve cells(*12*). Injection pain is caused by needle injury, distension, and inflammation due to percolation of the injected fluid and mucosa irritation in response to the anesthetic (*13*). Needle fear and phobia, due to injection pain and anxiety, may inhibit patients from receiving necessary dental treatment and worsen their oral health conditions (*2-5*).

The NFLJI systems are rarely used in dental practice, mainly due to the inconsistent outcomes (*14-16*) and unpredictable complications, such as bleeding and discomfort, unpleasant taste, and pain (*16, 17*). Furthermore, there is no clear guideline on how to perform NFLJI anesthesia. This knowledge gap leads to controversial clinical outcomes and complications. As the mechanism of NFLJI is different from that of needle injection and the outcome of NFLJI is affected by the NFLJI parameters, its techniques such as injection angle and NFLJI parameters need to be modified based on the mechanism and the anatomical structures of the injection sites.

We hypothesis that by adjusting the injection angle and NFLJI parameters based on the NFLJI fluid dynamics in the dentoalveolar region, we can develop the optimal technique for dental infiltration of nerve block anesthesia with desired efficacy and minimal complication.

This study aims to develop the optimal techniques for NFLJI dental anesthesia *in vitro*, namely local infiltration and mental incisive nerve block; to validate these techniques on cadavers; to evaluate the feasibility and safety of NFLJI in clinical practice.

Chapter 2: Literature Review

2.1. Pain

Pain is a complex constellation of unpleasant sensory, emotional and cognitive experiences (*18*). The nociception and perception of pain are commonly evoked by pressures and temperatures that may injure tissues, or by toxic molecules and inflammatory mediators (*18*), and manifested by certain autonomic, psychological, and behavioural reactions (*19*).

The most common stimulus for persistent pain is tissue damage, which is caused by trauma or surgery. Tissue damage is a mechanical noxious stimulus that can activate nociceptors which are pain-sensing nerve cells (*20*). These nociceptors can transmit information to the spinal cord dorsal horn or its trigeminal homolog, the *nucleus caudalis*, and ultimately to the brainstem cerebral cortex, where the perception of pain is generated (*20*) (*21*).

Perioperative pain management is essential for patients who receive invasive surgical or dental procedures and experience acute pain (*22*). Poorly managed acute pain can result in prolonged recovery (*23*), increased catabolism, increased cardiorespiratory work, immunosuppression(*24*), and coagulation disturbances(*25, 26*).

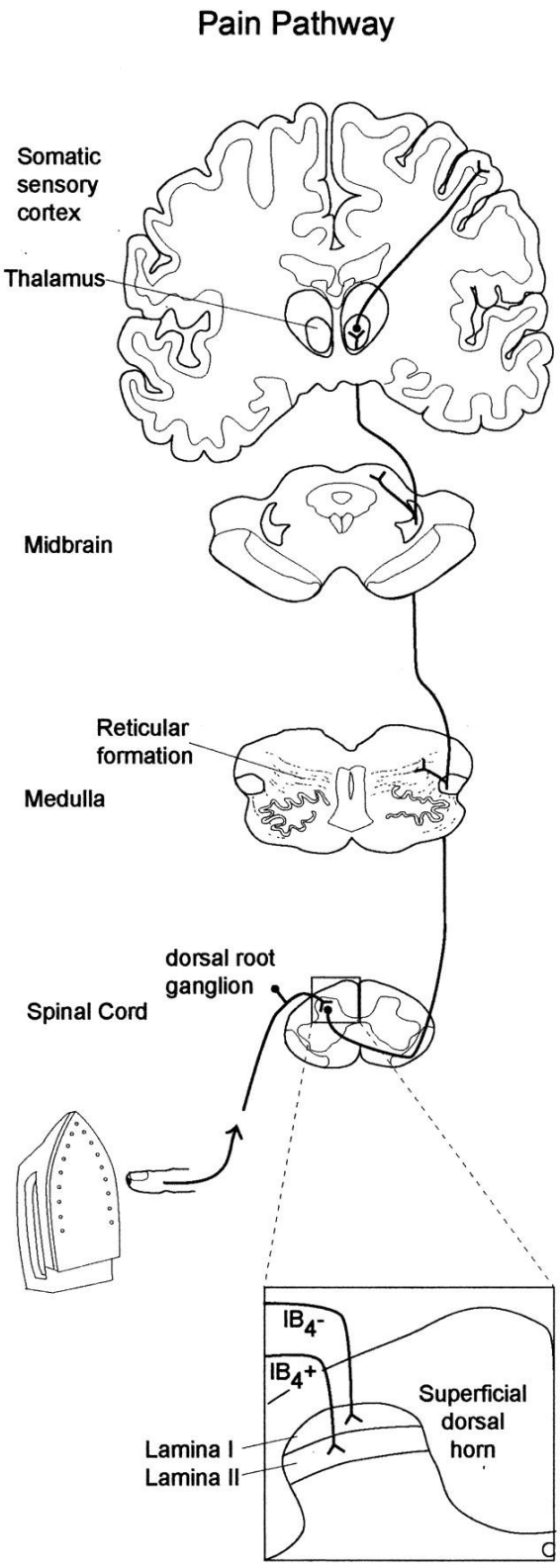

Figure 2.1 Mechanism of Pain (*21*). Reproduced with permission. Copyright (2001) National Academy of Sciences

2.2. Local Anesthesia

2.2.1. Chemical structure of anesthetics

Local anesthetics (LAs) have been studied for over one hundred years since cocaine was first discovered in 1860. LAs have an aromatic ring, an amine terminus, and an intermediate chain (-COO-R-or –CONH-R-) (*27*). The aromatic rings are lipophilic, which improve lipid solubility of LA and can be enhanced by aliphatic groups. The amine terminuses are hydrophilic. The intermediate chains define whether LA is an amino-ester or an amino-amide. Commonly used amino-ester LAs include cocaine, procaine, tetracaine, chloropropane, and benzocaine. Commonly used amino-amide LAs include lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine and levobupivacaine. The amino-esters are metabolized by blood and tissue esterases, while the amino-amides are metabolized by the liver's mixed-function oxidase system (*27*).

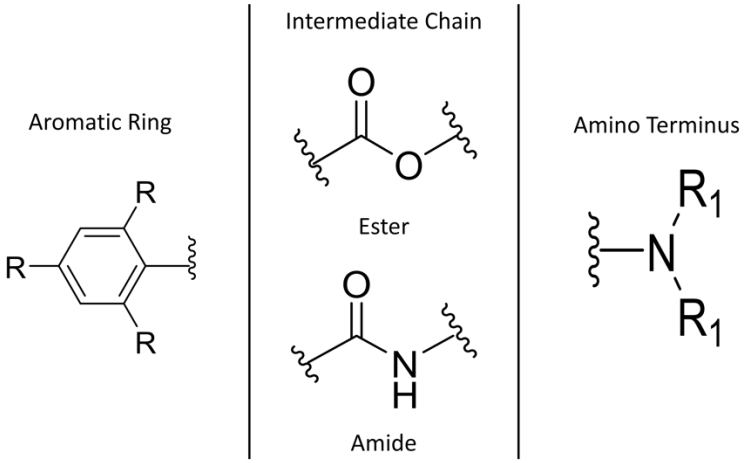

Figure 2.2 Structure of Local Anesthesia, made by Chemdraw 2018.

2.2.2. Mechanism of anesthesia: Blockage of sodium channels in nerve cells

LAs can systemically or locally block the pain signal transduction between pain nociceptors and the brain to prevent acute pain (i.e. perioperative pain) and chronic pain (i.e. neuropathic pain) (*27*). LAs work by binding and blocking the voltage-gated sodium channels in

the nerve cell membrane. Voltage-gated sodium channels initiate action potentials in nerve, muscle, and other excitable cells (*28*). The gating of voltage-sensitive Na+ channels determines the time course of the rising phase of the action potential and the length of the refractory period in the nerve, skeletal muscle, and heart (*28*). LAs can block the sodium channels by binding with the receptor site, formed by amino acid residues in the S6 segments in domains I, III, and IV (*28*).

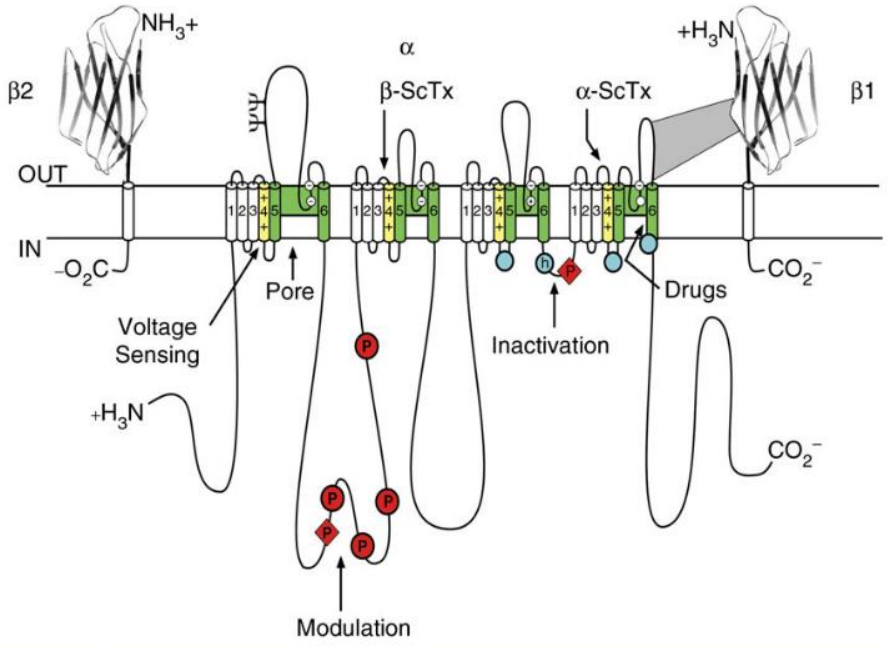

Figure 2.3 the primary structure of the subunits of the voltage-gated sodium channels (*28*). Reproduced with permission. Copyright (2013) John Wiley and Sons.

2.2.3. The physical-chemical properties and the effect of LA

The effects of local anesthetics are impacted by dose, time and type (*29, 30*). The dose can be increased by increasing the LA volume or concentration, and it leads to higher efficacy and prolonged anesthesia duration (*31*). The anesthesia effect will reduce along time as the body metabolize LA molecules. The effect of the type of anesthesia depends mainly on three factors:

the ionization constant(pK_a), the lipid solubility and the degree of protein binding (*12*). These three factors determine the anesthesia onset, potency and duration.

The pK_a determines the onset. The pKa of a molecule represents the pH at which 50% of the molecules exist in the lipid-soluble (uncharged) form and 50% in the water-soluble (charged) form (*30*). Only the uncharged form of the LA can cross the cellular membrane, whereas intracellularly, the drug needs to be conjugated to a hydrogen ion before it can bind to the local anesthetic receptor (*27*). The pKa of all local anesthetics is greater than 7.4 (physiologic pH), and therefore more than 50% of the molecules exist in the water-soluble form (*32*). Drugs with a lower pKa possess a more rapid anesthesia onset than those with a high pKa (*33*). For example, lidocaine has a pKa of 7.7 at 36°C (*12*), if it is injected into tissues with a physiologic pH of 7.4, then 52% of the LA molecules would exist in the charged form, and the other 48% would exist in the uncharged form that would be able to penetrate the neuron membrane.

$$\log\left(\frac{charged\ form}{uncharged\ form}\right) = pKa - pH$$

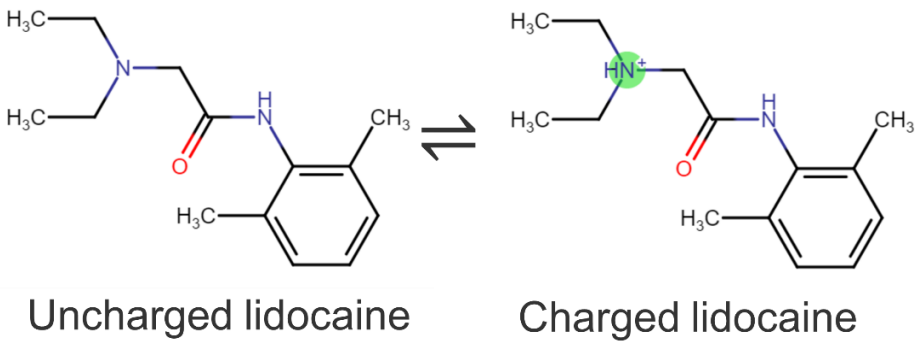

Figure 2.4 example of uncharged and charged lidocaine molecules.

The lipid solubility, determined by the aromatic ring and its substitutions, influences the LA potency (*30*). This solubility is evaluated by partition coefficients (O/B PQ) with H-octanol/buffer at pH 7.4 and 25°C. Great potency allows LA to penetrate the lipid cell membrane

more easily (*12*). For example, bupivacaine is more lipid-soluble and potent than articaine, allowing it to be formulated as a 0.5% concentration rather than a 4% concentration (*30*).

The protein binding degree determines the duration of anesthesia (*12*) (*30*). After penetration of the nerve cell membrane, a re-equilibrium occurs. The uncharged-form LA becomes the charged-form LA. Then the charged RNH^+ ions bind at the receptor site. The stronger the protein binding ability of the LA, the longer the anesthesia lasts. For example, bupivacaine has a strong protein binding degree of 95% while lidocaine has 65%; this makes bupivacaine the longest acting of the local anesthetics available in dental cartridges (*12*). Table 3.1 summarized the physical, chemical and pharmacokinetic properties of main LAs used in the clinical setting.

2.2.4. Delivery of local anesthetics

Local anesthetics can be used to treat acute pain (e.g., perioperative pain) or chronic pain (e.g., cancer-related pain)with one single injection or a control release formula (*34*). Many LA delivery techniques using needles injections have been developed and used for more than 100 years: injection into the tissues of the operation region (infiltration anesthesia); or on a specific peripheral nerves leading to anesthesia of all downstream structures (peripheral nerve blocks); or around the spinal cord (spinal and epidural blocks) (*34*).

In addition, many novel delivery techniques have been developed in the past decades (*35*) for control release formulas, including but not limited to nanoparticles (PLGA, lipid-polymer hybrid), microspheres (PLGA), liposome particles, and cements (CaP). These techniques can prolong LA release from 0.5-8 hours to 2-144 hours (*35*) or release the drug on-demand via ultrasound (*36*) or light (*37*).

2.2.5. Dental local anesthesia

There are two major types of local anesthetic injection techniques in dentistry: local infiltration and nerve block.

Local infiltration in dentistry works by depositing LA near the tooth region to block the small nerve terminals of the tooth apices and the surrounding soft tissue (Fig 2.5). Technically, local infiltration in dentistry is a small field block since it blocks the dental pulp and soft tissue distal to the injection site (*12*). Infiltration anesthesia can adequately anesthetize the maxillary teeth and mandibular anterior teeth due to the loose or thin bone structure (*38*). However, it cannot adequately anesthetize the mandibular posterior teeth since the nerves in that region are protected by denser or thinker bone (*38*). Therefore, a nerve block technique is needed for those cases.

Nerve block anesthesia is used to block the pain feeling of a larger operation regions by depositing LA near the main nerve bundle, which sometimes is not at the operation region (*12*). The nerve bundle controls the pain signal transfer from the downstream small nerve branches that control the soft tissue and tooth at the operation region (Fig 2.5). Common dental nerve block techniques include the inferior alveolar nerve block, the mental nerve block, and the infraorbital nerve block.

When the two major dental anesthesia techniques fail, other techniques are available, such as the periodontal ligament injection, and the intraseptal, intracrestal and intraosseous injections. However, these techniques are challenging to perform, and sometimes require special equipment.

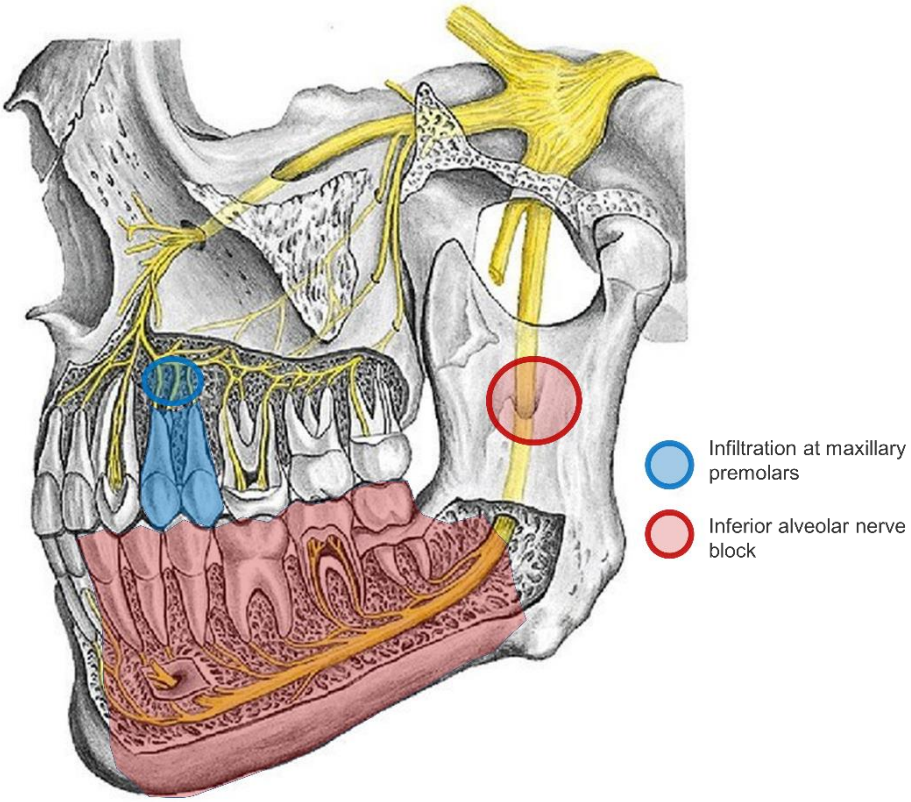

Figure 2. 5 Demonstration for the injection site and anesthetized region for infiltration(blue) and nerve block(red) anesthesia in the craniofacial region. Figure reproduced and adapted with permission. Grey's anatomy, 40th edition, Infratemporal and pterygopalatine fossae and temporomandibular joint, p545. Copyright (2008) Elsevier.

2.3. Pain from local anesthesia administration

Although short-lived, LA administration pain is severe enough for some patients to decline future surgery(*39*). Pain could be caused by three major factors: thermal, mechanical, chemical noxious stimuli (*40*). In addition to the above-mentioned biological factors, the "biopsychosocial model" of pain also emphasizes the role of psychological and social factors (*41*). More specifically, the anesthetic injections pain is caused by factors, such as the irritation of the mucosa from the anesthetic formulation(chemical), cold anesthetic solution(thermal), sensitivity of the injection site (biological), mechanical trauma caused by piercing the tissue(mechanical), and distension resulting from injecting the contents of the syringe(mechanical) and fear or anxiety of the needle(psychosocial) (*13*), (*39*), (*42*).

There are many solutions to minimize the LA administration pain, addressing the factors mentioned above. For example, selecting a proper buffer solution, warming the anesthetics before injection, injecting slowly, using small volume, choosing fine needles to minimize mechanical pain, using topical anesthesia before injection, educating patients with reassurance, distracting patients during injection(*42*) (*39*). However, none of the solutions above could eliminate needle injection pain, besides these increase the preparation time and cost in the clinic (Table 2.2).

In the table below, we summarized the cause of LA administration pain according to the biopsychosocial pain model(*40, 41*)

Table 2.1: Mechanism of LA administration pain(*13*), solution(*39, 42*), and limitations

Pain mechanism	LA injection pain	Solution	General Limitation
Chemical	Irritation of mucosa from anesthetic formulation	• Buffering the formula	• Time cost • Preparation cost • Higher failure rate if use small volume. • Risk of needle fracture
Mechanical	Mechanical trauma caused by piercing the tissue	• Fine (27-30gauge) needles • Longer (>1 inch) needles • Inject from wound edge or subcutaneous fat. • Insert perpendicular to the skin.	
Mechanical	Distension resulting from injecting drug	• Smallest volume • Slow injection • Inject from 'looser' subdermal. • Multi step, multi spot injection • minimize needle movement.	
Thermal	Cold anesthetic solution	• Warming the anesthetic to 37~42°C	
Biological	Sensitivity of the injection site	• Topical anesthesia prior to injection • Nerve blocks	
	Metal allergy	• NA •	
Phycological/Social	Needle phobia, anxiety	• Education • Reassurance • Distraction	

2.4. Needle phobia and avoidance behavior

Needle phobia, also called needle fear, is characterized by the American Psychiatric Association Diagnostics and Statistical Manual of Mental Disorders (DSM-IV) as the presence of fear, anxiety, and the occurrence of avoidance behavior (*43*) (*3*). A recent meta-analysis showed that 20-50% of adolescents and 20-30% of young adults experience needle phobia, and thus 16% of adult patients and 27% of hospital employees avoid influenza vaccination because of fear from needle(*44*). Hospitalized children report needle procedures as one of their most feared and painful experiences (*45*) (*46*).

A survey for dental patients showed that needle phobia presents in 11-19% of children (*4*). Needle-phobia and fear in dental clinics are also highly associated with avoidance behavior (*5*), which leads to profound health, dental, societal, legal implications and severe psychological, social, and physiologic consequences, requiring the dentist to exhibit compassion and respect(*3*).

Needle phobia results from a combination of genetic and life events, which means it could be inherited or learned(*43*) (*3*). The management of needle fear and phobia has been focused on managing the fear and pain associated with needle injections (*2*).

2.5. Needle-Free Liquid Jet Injection system

2.5.1. Different needle-free drug delivery systems

To solve the problems mentioned above and to improve patients' experience, needle-free injection systems have been developed. The needle-free cutaneous drug delivery techniques include liquid jets, powder jets, ultrasound, patch, microneedle(*47*) (*1*). Among these techniques, the needle-free liquid jet injection (NFLJI) system is the most cost-effective alternative to needle injection because it can accommodate existing commercial formulas designed for needle injection of anesthetics (*1*).

Needle-free liquid jet injection(NFLJI) systems work by creating a high-speed stream of fluid impacts the skin and delivers drugs across the skin into the subcutaneous or intramuscular region (*48*) (*1*). They could be powered by spring, laser, or energy such as gas or shock waves(*49*).

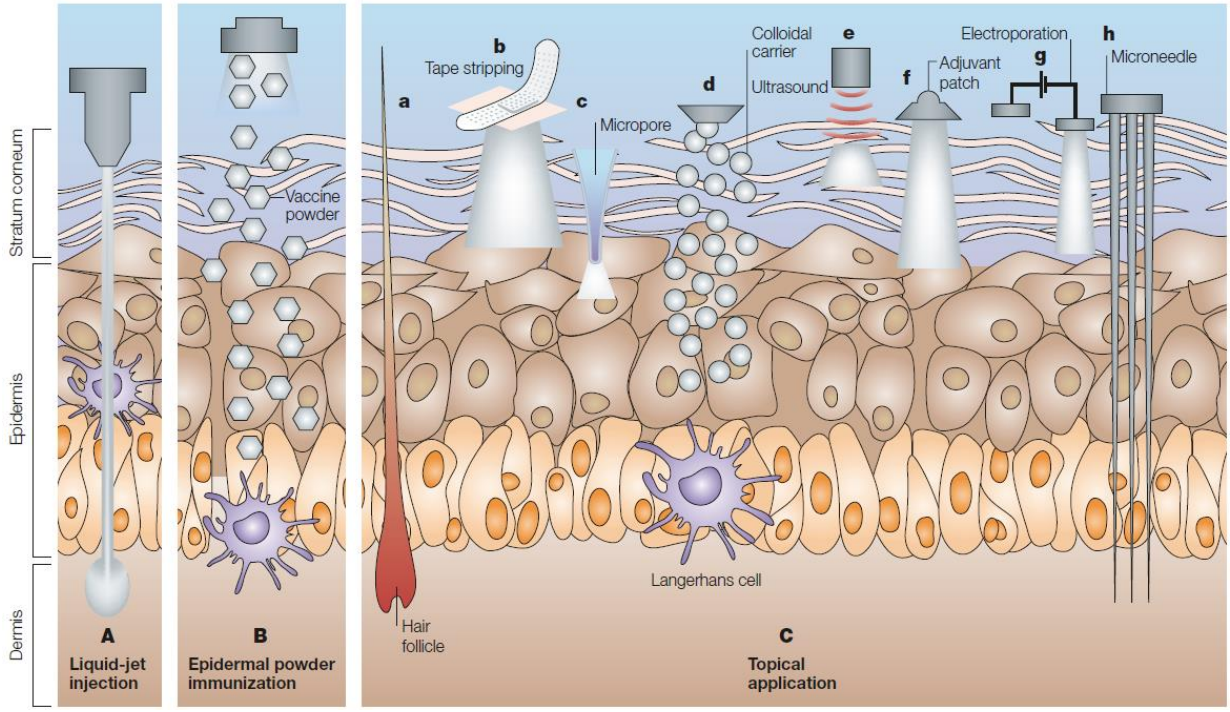

Figure 2.6 Needle-free castaneous routes delivery systems (47). Reproduced with permission. Copyright (2005) Springer Nature.

2.5.2. The advantages of NFLJI

The NFLJI systems provide a great alternative option for those patients with needle phobia since they work without a needle. Besides, they could avoid complications caused by needle injection (*12*, *50*), such as needle trans-infection, accidental needle stick injury for nurses or clinicians, needle fracture inside the tissue, and metal allergy. Moreover, the NFLJI could reduce the associated cost for medical sharps disposal, preparation, needle production and transportation.

NFLJI has been reported to provide lower injection pain (*14*, *51-53*), presumably because of the micro-level diameter orifice(76-360μm)(*1*), fast injection time (<0.3s), and high-velocity jet(typically >100m/s) that could minimize the mechanical pain stimulus.

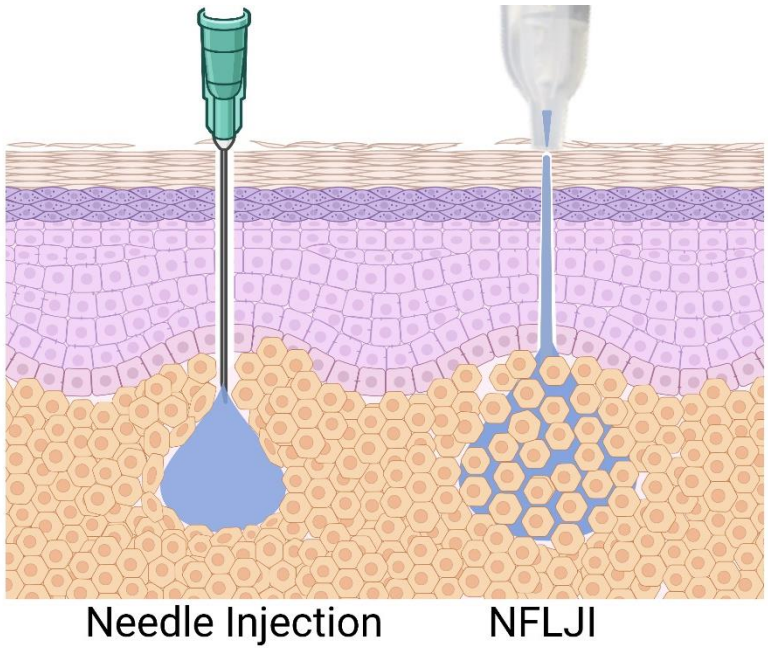

Figure 2.7 Demonstration of dispersion effect between needle injection and NFLJI, created with BioRender.

2.5.3. The applications of NFLJI

Needle-free liquid jet injection (NFLJI) systems are successfully and widely used in dermatology (*7*) and vaccination(*8, 9*) (*54*). Besides, many other applications have been investigated , including delivery of local anesthetics(*55*), growth hormone(*11*) or insulin (*56*) (*10*), corticosteroid, bleomycin, 5-ALA, botox(*57*), and stem cells(lipid cell). There are also potential applications still in the early stage, such as delivery of PRP (platelet-rich plasma), DNA molecules, particles encapsulated with a therapeutic drug. (Table 2.2 summarized all these applications)

Table 2.2 Application of NFLJI.

Application	**Therapeutic agents**	**Injection site**	**Penetration depth**
Anesthesia	Anesthetics e.g., Lidocaine, Bupivacaine	Oral mucosa; Surround tumor/moles/tags; Circumcision; Nail psoriasis	Infiltration 3-10 mm Nerve block: 5-20 mm
Vaccination	Vaccines	Arm	Intramuscular, 5-10 mm
Daily drug administration	Hormone, growth factors Insulin	Belly or arm	Subcutaneous, 3-5 mm
Dermatology	Botox	hand, face, feet, back, varies	Subcutaneous, 3-5 mm
	PRP	scalp, face,	Subcutaneous, 3-5 mm
	Corticosteroids	Nail psoriasis; Hypertrophic; scar/keloids; skin necrosis.	Intralesional, 3-10 mm
	bleomycin	Wart (varies sites)	Intralesional, 3-10 mm
	5-ALA	basal cell carcinoma sites	Intralesional, 3-10 mm
Others	Proteins/macromolecules lipid-derived stem cell Gene/DNA molecules Microparticle/ Nanoparticle	Specific tissue, organ, tumors	Varies

2.5.4. The disadvantages and challenges of NFLJI

Though NFLJIs have been developed for more than 50 years, they have not reached their full potential(*1*) due to many reasons. First, the techniques for NFLJI are poorly investigated; the injection technique is complex, it needs specialized training and equipment maintenance(*56, 58*). Second, the NFLJI is not applicable for the intravenous route(*56*). Third, even though NFLJI has proven its efficacy and safety in vaccination (*9*) (*54*) and dermatology (*7*) applications, its efficacy and safety in anesthesia or other application remain unclear.

2.6. The clinical safety and efficacy problem of NFLJI anesthesia

Previous clinical trials stated that though the injection pain of NFLJI is significantly less than conventional needle injection(*14, 59*), it could be a good alternative for conventional needle anesthesia (*60*), and it is more preferred among adults (*14*).

However, the efficacy and safety of NLFJI remain unclear and are poorly investigated (*14, 59*), and its efficacy is controversial (Table 2.3). Moreover, the use of NFLJI in dental anesthesia can result in complications such as bleeding and discomfort, unpleasant taste, and pain (*16, 17*), which raise safety concerns. Until now, many clinical trials for needle-free anesthesia still did not describe how they perform the injection, while local anesthesia's success rate is significantly influenced by the operator's performance (*61*). Therefore, a reproducible, effective, and safe injection technique for NFLJI dental anesthesia is needed.

The dentoalveolar region offers a unique set of challenging conditions for NFLJI due to the presence of hard bones underlying the thin layer of soft tissues. Therefore, understanding how liquid jet behaves in the dental alveolar region could help developing the optimal technique for NFLJI dental anesthesia.

Injections with NFLJI are very different from injections with needles. Needle injection work by piercing a sharp hollowed needle into tissue by force to create a pathway, then deliver the drug by pushing the syringe. The drug delivery distance depends on the needle length and injection spot. The needle-free injection works by creating a high-speed liquid jet that pierces the skin with an initial impact, then the drug is delivered by pressure through the micro wound and disperses underneath the tissues.

Table 2.3 Literature review of NFLJI dental anesthesia clinical studies.

Ref.	Design (procedure, sites)	Patients (sample size)	NFLJI	Agent	Method of injection	Main Outcomes (NFLJI vs. needle)
(*62*)	Two arm design (infiltration, NA, NA)	Adult (n=22)	INJEX	0.3mL 2% lidocaine with epinephrine	Perpendicular injection	Efficacy is equal (data not reported) Time of onset is similar (data not available) Duration of anesthesia is shorter (data not available) *
(*17*)	Split-mouth design (infiltration, anterior teeth, and upper molars)	Children (age 6-11) (n=87)	INJEX	0.4mL articaine 4% with epinephrine (used topical gel)	NR	Acceptance or Preference are reduced (12.6% vs. 73.6%) * Effectiveness is reduced (additional anesthesia 80.5% vs. 2.3%) * Pain during anesthesia is greater (70.1% vs. 4.6%) * Fear during anesthesia is increased (81.6% vs. 13.8%) * Complications are increased: bleeding (60.9% vs. 26.4%) *; bad taste (56.3% vs. 9.2%) *; stinging (46.0% vs. 6.9%) *; discomfort (18.4% vs.9.2%) *
(*14*)	Split-mouth (infiltration, premolar and molar region)	Adult (n=20)	MadaJet	0.4 mL 2% lidocaine with 1:80,000 epinephrine (used topical gel)	NR	Acceptance or Preference is greater (70% Vs 20%) * Efficacy is equal (EPT results were not reported) Pain is reduced (1.65± 0.93 vs 3.55± 1.67) * Fear is reduced (5.15± 3.18 vs. 1.6± 1.6) * Discomfort during injection is greater (2.55± 1.35 vs. 0.55± 0.76) * Time of onset is faster (21± 6.20 vs.48.2± 20.85s) * Duration is shorter (20.75 ± 3.53 vs 50 ± 9.32 min) * Bad taste tends to be greater (1.8± 1.36 vs.1± 1.45)
(*15*)	RCT, split- mouth (infiltration, maxillary first molar)	Adults (n=41)	Comfort-in	1mL Lidocaine 2% with epinephrine 1:100,000	Perpendicular injection	Pain score during injection is similar [12.2(0-55.4) Vs 12.1(0-53.8)] Latency time for anesthesia is similar (2 vs. 2 min) Duration of pulp anesthesia is shorter (Mean: 20 vs. 40min) * Efficacy is equal: no patients required additional anesthesia
(*16*)	Split-mouth (infiltration, premolars)	Adolescent orthodontic patient(n=28)	INJEX	0.4mL 4% articaine with 1:200,000 epinephrine	Perpendicular injection (3000psi)	Pain score during injection is reduced (1.5± 1.8 vs 3.14±2.01) * Pain score during procedure is grater (3.86± 3.23 vs 2 ±2.05) * Efficacy is reduced (additional injection 28.6% vs .1%) * Bad taste is greater (3.36 ±2.38 vs 2.11± 2.18) * Duration is shorter (40.89±17.32 vs 64.46±27.93 min) *

INJEX: INJEX Pharma, Berlin, German; MadaJet: MADA Medical Products Inc, New York, USA; Comfort-in: Mika Medical Inc, Busan, Korea.

* means $p<0.05$, there was a significant difference of this variable among needle-free and needle group. NA, information is not available in the paper.

2.7. Mechanistic consideration for NFLJI

Previous studies showed that NFLJI tissue penetration and dispersion depend on three aspects: 1) the NFLJI (supply pressure, volume, orifice diameter) (*63*), 2) the soft tissue(Young's modulus)(*64*), 3) the injected fluid (viscosity and density)(*65*). Among these, NFLJI parameters, such as pressure and volume, are of interest because they can be adjusted according to the injection site and fluid to optimize the injection outcome.

These NFLJI parameters control the energy and velocity of the liquid jet exiting the nozzle, and subsequently control the jet penetration and dispersion inside the soft tissues. Hence a higher supply pressure or volume results in deeper penetration. A previous study injecting methylene blue (5%,0.2mL) into cadaver skin using a supply pressure of 6-8.5 bar (87-123psi) found that higher pressure resulted in deeper penetration depth (Seok et al. 2016). Similarly, another study injecting dye (1.0-2.5ml) into ballistic gelatin (10%w.t.) using a supply pressure of 40-200 MPa found that pressure influenced the depth of penetration, but not the dispersion profile, while the increased volume increased both the penetration depth and the dispersion (Grant et al. 2015)

Injecting fluid with lower density or viscosity create deeper jet penetration. A previous study found that compared to the more viscous and dense latex fluid (density 1.10g/mL, viscosity 557.1g/m·s), NFLJI using an aqueous solution 5% methylene blue (density 1.01g/mL, viscosity 13.1g/m·s) can penetrate deeper into the skin of a cadaver (Seok et al. 2016).

The mechanical properties of the tissues have a strong influence on jet penetration(*1*), namely their Young's modulus and fracture toughness. The Young's modulus is the mechanical property of a material that resists force and deformation(*66*). Increased Young's modulus of the materials results in a decrease in penetration depth(*64*). Fracture toughness is the ability of a

material to resist fracture(*67*). Increased fracture toughness of the material will require higher liquid jet energy to create a crack, thereby decrease the penetration depth. No study has investigated the relationship between fracture toughness of the material and jet penetration.

Chapter 3: Delivery systems for local anesthetics

3.1 Preface:

In this chapter, we discussed the delivery system for local anesthetics, the material, the formulation, *in vitro* and *in vivo* effect and safety for post-operative pain management, especially for bone surgery.

This chapter(*68*) has been published in the journal “Drug Discovery Today.

Dupleichs, Manon, Qiman Gao, Zahi Badran, Pascal Janvier, Jean-Michel Bouler, Olivier Gauthier, Faleh Tamimi, and Elise Verron. "Delivery systems of local anesthetics in bone surgery: are they efficient and safe?." Drug discovery today 23, no. 11 (2018): 1897-1903.

https://doi.org/10.1016/j.drudis.2018.06.019

Delivery systems of local anesthetics in bone surgery:

are they efficient and safe?

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Article type: Review Article

Highlight:

- Postoperative pain following bone surgery is a frequent concern.
- Management of postoperative pain includes administration of local anesthetics.
- Optimized local anesthetic delivery systems should prolong duration of analgesia.
- High levels of local anesthetics affect tissue regeneration.

3.2. Abstract

Management of postoperative pain following bone surgery includes administration of local anesthetics (LAs). Smart delivery systems, including triggered systems, have been designed to provide a continuous release of LA *in situ*. However, these systems can provide a high level of LA locally. This review will examine the state-of-the-art regarding the LA delivery systems optimized for management of postoperative pain in bone surgery and will discuss the potential adverse effects of LAs on the overall pathways of bone healing, including the inflammation response phase, hemostasis phase, tissue repair phase and remodeling phase. There is a clinical need to document these effects and the potential impacts on the clinical outcome of the patient.

3.3. Introduction

Postoperative pain following bone surgery is a frequent concern. The severe pain caused by surgical interventions involving hard tissues can jeopardize treatment success, and compromise patient recovery, mobility, function, quality-of-life and autonomy, as well as prolonging hospitalization (*69, 70*). This postoperative pain can become chronic and therefore more difficult to manage. There is a potential connection between pain, inflammation, and the healing process after bone surgery through the immune system. Consequently, pain management during the first four postoperative days should be as efficient as possible to minimize the risk of developing chronic pain and thus compromising the healing process. Postoperative pain after bone surgery is usually managed with systemic administration of conventional analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids (*71*). In addition to adverse effects induced by analgesics, their prescriptions increase the overall intervention costs, thus adding an economic burden on health system expenses (*72*). In contrast to this systemic approach, local analgesia through peripheral nerve blocks is very promising because it can limit postoperative pain while avoiding complications and limitations of systemic drugs (*73, 74*). Unfortunately, therapeutic efficacy is largely compromised by a short effective duration. For this reason, delivery systems of local anesthetics (LAs) have been developed to achieve continuous analgesia.

3.4. Management of postoperative pain with local anesthetic drugs

Since the introduction of cocaine, various LAs have been adopted in clinical practice to manage postoperative pain(*75, 76*). Although binding to the sodium channel through the hydrophilic pathway is the main mechanism of action of LAs(*27*), alternative pathways described for uncharged LA (e.g., benzocaine) or, by contrast, for permanently charged LA (e.g., lidocaine derivative QX-314) also exist(*77*). Uncharged LA can pass through the nerve membrane and

reach the lateral fenestrations in the sodium channel. By contrast, influx of QX-314 seems facilitated by the formation of a large pore in response to stimulation of the transient receptor potential vanilloid 1. Table 1 summarizes physical–chemical and pharmacokinetic characteristics involved in the LA activity (*27*).

Table 3.1 Physical, chemical and pharmacokinetic properties of main LAs used in clinical setting (amino-ester and amino-amide) (27)

		Physical-chemical					Pharmacokinetic		
		ionization constants (onset)	Lipid solubility (potency)	Protein binding (duration)					
	Drug	**pKa**	**O/B PQ[a]**	**PB %**	**MW (g/mol)**	**t1/2 (h)**	**Clinical Duration**	**Max dose (mg/kg)**	**With Epinephrine**
Ester	Benzocaine				165				
	Cocaine	8.7(slow)	(moderate)		303		0.5-1	3	
	Chloroprocaine	8(fast)	(moderate)	(short)	271		0.5-1	11	14
	Procaine	8.9(slow)	1.7(weak)	6(short)	236	0.1	0.5-1	12	
	Tetracaine	8.5(slow)	221	76(long)	264		1.5-6	3	
Amide	Articaine	7.8(fast)	17(moderate)	70(short)	321	0.5	0.5-1.5	4	7
	Bupivacaine	8.1(slow)	346(potent)	95(long)	288	3.5	1.5-8	2.5	3
	Lidocaine	7.9(fast)	2.4(weak)	64 (moderate)	234	1.6	0.75-1.5	4.5	7
	Mepivacaine	7.6(fast)	21(moderate)	77(moderate)	246	1	1-2	4.5	7
	Prilocaine	7.9(fast)	25(moderate)	55(moderate)	220	1.6	0.5-1	5-7	7-8.5
	Ropivacaine	8.1(slow)	115(potent)	94(long)	274	1.9	1.5-8	3	3.5

Abbreviations: PB, protein binding; MW, mass weight.
[a] Partition coefficient were measured between oil and buffer (O/B) at pH 7.4 and 25°C

Unfortunately, therapeutic efficacy is largely compromised by a short duration of action (Table 1). To overcome this limitation, continuous and controlled administration of LA at surgical sites or around nerves that innervate the site is often used by surgeons. This local administration can extend analgesia to better prevent chronic pain with limited systemic effects. For example, femoral nerve block (FNB) has been shown to (i) reduce the postoperative need for opioids after total knee arthroplasty (TKA), (ii) reduce hospital stays of patients and (iii) increase ability to undergo physical therapy compared with patients receiving oral analgesia postoperatively. However, this technique can be associated with a risk of decreased muscle tone of the quadriceps, which counteracts effective rehabilitation and increases the risk of patient falls(*78*). Local infiltration analgesia (LIA) is an alternative regional anesthesia method for the control of acute postoperative pain following knee and hip replacement surgery. Patients who received local periarticular injection of ropivacaine, ketorolac and epinephrine showed lower pain levels as compared with those who received FNB(*79*). Despite these clinical benefits, the efficacy of LAs is still limited by the short duration of analgesia. With a view to prolonging LA duration without compromising patient safety, different options have been envisaged including drug delivery systems, structural modification of LA molecules and coadministration of vasoconstrictors. Among them, prolonged-release formulations of LA using biocompatible drug carriers (Table 2) have been designed to remain at the site of injection and release LA slowly over time at a therapeutic dose. These systems should be easily and simply administered to patients.

Table 3.2 Description of characteristics required for a LA delivery system.

Clinical efficacy	Protection of local anesthetic (LA)
	Solubilization of LA
	Sustained release of LA
	Prolonged duration of nerve block
	Favorable ratio of sensory and motor blocks
Safety	Biocompatible
	Biodegradable
	Avoiding high local level of LA
	Minimal local inflammation response
	Absence of neurotoxicity
	Absence of myotoxicity
	No systemic toxicity
	Stability of formulation
Administration formalities	Easy to administer to patients
	Initiated by a single administration
	No need of general anesthesia or surgical procedure
	No need of sophisticated materials
Manufacturing process	Cost effective
	Easy to produce
	Industrial scale-up

3.5. Formulation for optimized delivery of local anesthetics

3.5.1. Polymers

Hydrophobic polymers such as poly (lactic-co-glycolic acid) (PLGA) and PEG–polylactic acid (PELA) have been used to produce particles for controlled release of LA. Release profile can be optimized by varying the size of particles, drug content, polymer: drug ratio and excipient used. Administration of PLGA microspheres significantly prolongs the release of bupivacaine to 144 h, whereas plasma levels of bupivacaine were undetected 8 h after injection of bupivacaine solution (*80*). Wang et al.(*81*) investigated the analgesic effect of ropivacaine–PELA nanoparticles (10% w/w) on a postoperative pain model in rats. These nanoparticles increased the duration of sciatic nerve block over 3 days after a single administration, whereas systemic injection of ropivacaine was only effective for 8 h. This prolonged release could result in long-lasting local exposure of the nerve to ropivacaine with the metabolism of the PELA nanoparticles.

Recently, lipid–polymer hybrid nanoparticles (LPNs) have also been used to regulate the release of bupivacaine(*82*). LPNs consist of two major components: (i) the PLGA core capable of encapsulating hydrophilic and hydrophobic drugs; and (ii) single or multiple lipid layers (lecithin). By combining the characteristics of polymeric nanoparticles and lipids, LPNs provide high structural integrity, stability during storage and prevent the fast release of the drugs. Indeed, prolonged controlled release of bupivacaine from LPNs was observed for up to 96 h with only 19.3 ± 3.6% of the drug released at 10 h compared with 50.7 ± 3.1% of release at 10 h obtained with bupivacaine-loaded PLGA nanoparticles. Electrical stimulation on mice showed these LPNs increased the duration of analgesia by 5 h compared with bupivacaine-loaded PLGA nanoparticles.

Interestingly, chitosan (CS) has been included in LPN formulations to create a strong crosslinking complex of CS resulting in denser particles that can delay lidocaine release(*83*). As expected, its release was slower than that of the liposomal formulation. The release profile exhibits a biphasic pattern characterized by an initial burst-release of 40% of the lidocaine in 8 h followed by sustained release up to 72 h (vs 48 h for liposomes). This slower release of lidocaine results from its entrapment by the lipid matrix.

3.5.2. Liposomes

A liposomal bupivacaine formulation (Exparel®) was recently approved by the FDA in 2011 for postsurgical analgesia. Briefly, this multivesicular liposome contains a novel phospholipid excipient, dierucoylphosphatidylcholine, cholesterol and tricaprylin, which allows a particularly high capacity for bupivacaine loading (fivefold ratio compared with conventional preparations). After its injection into trochanter in rats, sciatic nerve block lasted 240 min as compared with 120 min for 0.5% (w/v) bupivacaine HCl and 210 min for 1.31% (w/v) bupivacaine HCl (*84*). A Phase II dose-ranging study on patients with TKA reported that a 532 mg dose extended the duration of local analgesia from under 12 h to 5 days. This therapeutic dose was well-tolerated, had a higher safety margin and showed a favorable safety profile compared with bupivacaine and control groups (*85*).

To date, several randomized clinical trials have shown that liposome bupivacaine periarticular injection can provide better postoperative analgesia compared with placebo or plain bupivacaine by periarticular injection or nerve block(*86, 87*). Liposome bupivacaine can reduce pain score and opioid analgesia consumption and shorten hospitalization in patients who under- went TKA (*88*). It might be able to improve postoperative physical performance of walking and stairclimbing, to reduce hospitalization cost and speed up postoperative recovery in TKA (*89*).

Recently, co-injection of liposomal bupivacaine with a co-delivery of two encapsulated adjuvant compounds, dexamethasone and dexmedetomidine, has been shown to enhance the duration of sciatic nerve block 2.9-times more than liposomal bupivacaine alone (*90*).

Currently, published data are still insufficient to establish a well-conducted comparison between liposome bupivacaine and various mixtures of non-opioid analgesia. Indeed, there is not enough evidence to support whether liposome bupivacaine is superior to a standard analgesic mixture, considering the aspects of pain relief, opioid consumption and hospital stay. Although liposomes have excellent properties for drug delivery, their use remains compromised by physical instabilities (size increase by vesicle fusion) and chemical instabilities (lipid peroxidation) during storage limiting their shelf life, sterilization and industrial scale-up.

3.5.3. Calcium phosphate bone substitutes

Calcium phosphate (CaP) biomaterials are extensively used for bone reconstructive surgery because they are biocompatible, bio-active and osteoconductive (*91*). Interestingly, they can act as local drug delivery systems(*92, 93*). The first combination of CaP with bupivacaine provided a dose-dependent analgesic effect during the first postoperative days (*94*). Lidocaine has been mixed with different kinds of CaP cement (CPC) components. The drug release depends on cement pH and composition and can be prolonged for up to 6 days (*95*). Salts of lidocaine, bupivacaine and levobupivacaine were incorporated into the solid phase of CPC (*96*). Cement released >60% of the lidocaine within the first 24 h, whereas bupivacaine or levobupivacaine reached 60% release after 144 h of incubation. Recently, a critical-size bone defect of rat femur was filled by an injectable CPC loaded with bupivacaine or ropivacaine (*97*). The functional evaluation of the gait performed with the CatWalk system demonstrated significant pain relief during the short-term postoperative period.

3.5.4 Smart controlled system

In the era of personalized medicine, LA delivery systems should achieve responsive and adjustable release according to the changing needs of patients in terms of timing, intensity, and duration of analgesia. In this attempt, three leading-edge external triggers have been conceived based on light, ultrasound and magnetic fields and could be placed on a nerve allowing the patient to achieve precise titration of LA (*98*). Because tissue penetration by light is dependent on its wavelength and power, relatively deep light penetration of tissue should be expected at near infrared (NIR) wavelengths (650–900 nm) up to 10 cm. Following irradiation, gold nanorods (GNRs) incorporated within liposomes can convert light energy to heat resulting in a phase transition of the lipid bilayer or pressure fluctuations that disrupt the lipid membrane. Subsequently, the ordered gel phase is transformed into a disordered liquid crystalline phase allowing the release of LA contained within the liposomes. After their implantation into a rat hind paw, irradiation with NIR light (808 nm) induced repeated infiltration analgesia (*99*). Interestingly, varying the irradiance and duration of irradiation can modulate the analgesic effect. However, NIR light can be significantly attenuated with progressive depth and increasing irradiance can induce severe tissue injury. Consequently, the formulation has been modified to render liposomes more sensitive to low temperatures(*100*). In fact, this new formulation was sensitive to low irradiance over short durations (1-2 min), which would be ideal to relieve pain as quickly as possible.

A photosensitizer contained inside liposomes produced singlet oxygen upon irradiation with NIR light resulting in peroxidation of unsaturated lipids in the liposome bilayer. Consequently, liposomes became more permeable and released encapsulated LA (*37*). In vitro release of LA reached 6% in response to NIR irradiation. Injection of these liposomes at the sciatic nerve of

rats provided an initial nerve block lasting 13.5- 3.1 h. Repeated periods of nerve block could be induced by NIR irradiation. The effective sensitivity to light of devices has been enhanced by co-delivering dexmedetomidine (*98*). As an α2-adrenergic agonist, dexmedetomidine induced local vasoconstriction maintaining a high local concentration of co-administered LA. Dexmedetomidine provided effective triggering with irradiation at 75 mW/cm^2 over 5 min compared with 330 mW/ cm^2 over 15 min without dexmedetomidine. Moreover, the threshold for providing nerve block was reduced from 76 J/cm^2 to 4 J/cm^2 with dexmedetomidine. Finally, dexmedetomidine enhanced the therapeutic effect of the released LA resulting in more nerve block events triggered (9 vs 2 without dexmedetomidine).

Unlike light, ultrasound is a common noninvasive technique that can be applied in a focused manner minimizing energy in surrounding tissue. Using parameters similar to those used in clinical imaging (high-frequency low-intensity ultrasound; HFLIU) seems to be safe(*101, 102*). Many of the current ultra-sound-triggerable drug delivery systems, such as micelles, liposomes, composites and hybrid materials, are responsive to the thermal and mechanical effects of ultrasound waves. Recently, smart liposomes containing sonosensitizer protoporphyrin IX have been shown to release reactive oxygen species (ROS) in response to ultrasound stimuli. Once released, ROS peroxidated the unsaturated lipids in the bilayers leading to the release of LA. For example, liposomes provided ~36 h of continuous initial nerve block on a rat sciatic nerve model (*36*). The nerve block duration depends on the extent and intensity of insonation. It would then allow an additional half-day off on-demand nerve block, enabling personalized narcotic-free pain management.

Based on previous studies demonstrating the efficiency of exogenous microbubbles to enhance drug flow through the skin(*103*), Cullion et al. have explored the positive effects of HFLIU in

conjunction with microbubbles on two LA-mediated nerve blocks (i.e., tetrodotoxin and bupivacaine)(*104*) (*105*). Their device markedly improved LA block frequency and duration of sensory and motor nerve block. For example, 25 mM tetrodotoxin in combination with HFLIU and microbubble treatment resulted in reliable nerve block, and 30 mM tetrodotoxin induced a nerve block greater than or equal to the duration achieved with 0.5% bupivacaine.

To summarize, these innovative triggerable drug delivery systems should achieve adjustable on-demand local anesthesia in terms of dose magnitude and timing. The dynamic range of release kinetics can be adjusted by changing the composition and geometry of the membrane to match the therapeutic window for optimized analgesia. However, efforts must be continued to provide excellent reproducibility and low off-state leakage. Once optimized, these devices based on continuous-wave laser systems or LEDs would be perfectly adapted for point-of-care systems, reducing the costs of health systems. Table 3 shows the main results obtained from biological studies.

Table 3.3 Summary of data obtained from in vitro and in vivo evaluation of LA delivery system.

***In vitro* studies**						
Authors	**Year**	**Materials**	**Formulation**	**Loaded LA**	**Duration of release**	**Refs**
Ma et al.	2017	PLGA hybrid	Nanoparticles	Bupivacaine	96 h	(*82*)
Wang et al.	2016	Lipid-polymer hybrid	Nanoparticles	Lidocaine	72 h	(*83*)
Wang et al.	2016	PLEA	Nanoparticles	Ropivacaine	3 days	(*83*)
Verron et al.	2010	CaP	Microgranules	Bupivacaine	24 h	(*94*)
Irbe et al.	2012	CaP	Cement	Lidocaine	6 days	(*95*)
Colpo et al.	2018	CaP	Cement	Bupivacaine Levobupivacaine	60% at 144 h	(*96*)
Dupleichs et al.	2018	CaP	Cement	Bupivacaine Ropivacaine	72% at 96 h 64% at 96 h	(*97*)
Rwei et al.	2017	Sonosensitizer	Liposome	Tetrodotoxin	7% at 2 h	(*98*)
Zhan et al.	2016	Gold nanorod	Liposome	Tetrodotoxin	10% at 10 min	(*99*)
Zhan et al.	2017	Gold nanorod	Liposome	Tetrodotoxin	2-19% irradiation	(*100*)
Rwei et al.	2015	Photosensitizer	Liposome	Tetrodotoxin	5.6% at 2 h	(*37*)
***In vivo* studies**						
Authors	**Year**	**Materials**	**Formulation**	**Loaded LA**	**Duration of anesthetic effect**	**Refs**
Schmidt et al.	2015	PLGA	Microshpere	Bupivacaine	144 h	(*80*)
Qi et al.	2016	PELA	Nanoparticles	Ropivacaine	3 days	(*81*)
McAlvin et al.	2014	PLGA	Liposomes	Bupivacaine	240 min	(*84*)
Rwei et al.	2018	Lipid mixture	Liposomes	Bupivacaine	16 h	(*90*)
Verron et al.	2010	CaP	Microgranules	Bupivacaine	72 h	(*94*)
Dupleichs et al.	2018	CaP	Cements	Bupivacaine Ropivacaine	>72 h >72 h	(*97*)
Rwei et al.	2017	Sonosensitizer	Liposome	Tetrodotoxin	36 h	(*98*)
Zhan et al.	2016	Gold nanorod	Liposome	Tetrodotoxin	5 h	(*99*)
Zahn et al.	2017	Gold nanorod	Liposome	Tetrodotoxin	62 h	(*100*)
Rwei et al.	2015	Photosensitizer	Liposome	Tetrodotoxin	24 h	(*37*)
Cullion et al.	2018	Lipid mixture sonication	Microbubbles	Tetrodotoxin	134 min	(*105*)

3.6. Biological drawbacks of high levels of LA

Regardless of the method or delivery system used, local release of high levels of LA could impact the short-time wound healing process. This could partially be the result of the deregulation of the initial inflammatory response and later tissue proliferation. The overall pathways of bone healing include inflammation response phase, hemostasis phase, tissue repair phase and remodeling phase. Consequently, there is a clinical need to document these effects and the potential impacts on the clinical outcome of patients.

3.6.1. Inflammatory response

LAs have been described to have anti-inflammatory properties during the main stages of bone healing (i.e., homeostasis, inflammation proliferation, differentiation). Despite the fact that the molecular mechanism of their anti-inflammatory effect remains unclear, several hypotheses have been proposed. For example, perioperative immunosuppression observed in surgical patients, as well as the proinflammatory and anti-inflammatory cytokines, synergistically increases its suppressive effects on the immune system(*106*). Also, LAs could modulate various steps of the inflammatory cascade including leukocyte adhesion, migration, activation and granulocyte phagocytosis. Furthermore, LAs have been shown to affect polymorphonuclear neutrophils (PMNs) directly, as well as macrophage and monocyte function in a dose-dependent and reversible manner(*107*). Ropivacaine and lidocaine (100–300 mM) decreased tumor necrosis factor (TNF)-a-induced upregulation of CD11b/CD18 surface expression on PMNs in vitro, thus leading to a decrease of PMN adherence, migration and accumulation at the site of inflammation(*106*).

Local tissue reactivity has also been assessed after injection of liposomes containing LA. Animals receiving liposomes showed mild inflammation at the injection site. Foamy

macrophages were observed at the injection site, showing particle uptake(*98*). The mild inflammation from liposome injections is generally considered safe(*87, 108*). Recently, safety concerns relating to the new generation of triggered drug delivery systems have been addressed. For example, insonation caused no significant inflammation when ultrasound parameters were similar to those used for therapeutic ultrasounds (*36*).

3.6.2. Tissue Injury

In vitro LAs have shown cytotoxic effects on muscular cells (*109*), fibroblast cell lines(*110*) and intervertebral disc cells(*111*). This could undermine neovascularization, fibroblast proliferation and collagen secretion, and could downregulate the proliferative stage of wound healing. Bupivacaine caused more myotoxic damage than levobupivacaine and ropivacaine in the skeletal muscle of rats (*109*).

Animals injected with liposomes loaded with LA showed mild inflammation at the injection site, whereas minimal inflammation was seen in the adjacent muscle (*98*) and no significant tissue toxicity was reported from liposomes(*87*). Moreover, special attention should be paid to the latest generation of triggered drug delivery systems. For example, animals treated with 1 MHz ultrasound for 5 min at an acoustic intensity of 0.1 W/cm2 had mild residual inflammation at 14 days and myotoxicity had resolved by 14 days(*105*). Similarly, there was no tissue toxicity either immediately after applying ultrasound at 3 W/cm2, 1 MHz, 10 min or in the following four days (*36*). Because ultrasound can have intrinsic effects on neuronal function, neurotoxicity of these triggered systems has also been evaluated. Previous studies using animal models of neuronal injury demonstrated that neuronal suppression secondary to acoustic waves is proportional to acoustic intensity administered, with focused high intensity (35 W/cm2) resulting in suppressed axonal conduction. Intensities of 390– 3000 W/cm2 generating nerve block that lasts for weeks,

and very high intensity (7890 W/cm2) causes almost complete axon degeneration (*112*). Fortunately, these intensities are orders of magnitude higher than those required by these triggered delivery systems. At lower ultrasound intensities, ultrasound induces nerve stimulation rather than suppression. Applying HFLUI did not induce nerve damage during a 2-week observation period following injection (*105*). Similarly, no significant neurotoxicity was observed in any animals receiving ultrasounds at high frequency (1 MHz, 3 W/ cm2) (*36*).

3.6.3. Hemostasis

Although it is well-known that LA infiltration causes vasoconstriction at low concentrations and vasodilation at high concentrations, the vasoactive effect varies depending on the drug used, because the latter determines either vasoconstriction or vasodilation. For example, prilocaine and mepivacaine are rather vasoconstrictory at clinical doses whereas lidocaine has vasodilator activity. Of the two enantiomers of bupivacaine, the S(-)levorotatory one seems the most vasoconstrictive. However, changes in the pulpal blood flow measured in patients treated with 0.5% levobupivacaine or 0.5% bupivacaine are not statistically different (*113*).

Also, vasoconstrictor molecules have usually been added to LA to effectively reduce drug absorption and toxicity, as well as surgical bleeding. Although a recent RCT found that, after total hip arthroplasty, LA with epinephrine infiltration did not significantly modify pre- and post-operative bleeding (*114*), LA infiltration has been shown to reduce bleeding after TKA. Hemostasis changes have been extensively studied following injections of various LAs ± epinephrine in patients undergoing dentistry surgery such as tooth extractions (*115*) (*116*, *117*). Data strongly suggest an absence of significant hemodynamic modifications regardless of the administration protocol used (concentration of LA, ratio of LA: epinephrine). Nevertheless,

further studies are still needed to address the related mechanisms affected by LAs to understand the mechanism of vasodilative and vasoconstrictive effects.

3.6.4. Osteoarticular regeneration

Effects of LAs on mesenchymal stem cell (MSC) activity have been studied because they can differentiate into a variety of cell types, including osteoblasts, chondrocytes, myocytes and adipocytes. MSCs probably play a crucial part in healing following surgical procedures such as microfracture and ligament reconstruction. Ropivacaine caused the fewest adverse effects on human MSCs, whereas lidocaine or bupivacaine seem to induce the most significant effects on MSC viability (*118*). Herencia et al. investigated the role of procaine in osteo/odontogenesis of rat bone marrow MSCs in *vivo* (*119*). They observed that procaine administration drastically reduces the mineralization and osteo/odontogenesis of bone marrow MSCs by inhibiting the Wnt/β-catenin pathway through the increase of Gsk3β expression and β-catenin phosphorylation. These effects of procaine were also observed on mature osteoblasts.

Chondrolysis is the irreversible destruction of previously normal articular cartilage, including the matrix and cellular element. Intra-articular injection of LAs increased risk of chondrolysis for patients after articular surgeries (*120*). However, potential associations, including high-flow intra-articular pain pumps, thermal devices, type of anchors and various sutures must be taken in consideration. A causal relationship between the infusion of LAs and the development of glenohumeral chondrolysis has been established (*121*). Results showed bupivacaine, lidocaine, ropivacaine and levobupivacaine are all toxic to cartilage in a dose-dependent manner. In summary, although these devices are bio-compatible, potential biological drawbacks as a result of high local levels of LAs highlight the need to control and optimize the release of LAs from the delivery systems.

3.7. Concluding remarks and future perspectives

Multimodal management of postoperative pain is based on the administration of LAs. One of the major challenges is to design a biocompatible and resorbable biomaterial capable of delivering LAs continuously at therapeutic doses *in situ* without damaging the surrounding soft tissues (Table 2). Even though the clinical efficacy of such smart delivery systems might be of high benefit in pain management, an objective comparison of results from different studies remains difficult owing to the various protocols and formulations used. Furthermore, interpretation of *in vivo* data is extremely complex because it depends on the animal model, the nerve blocks tested and the pain assessment methods. Indeed, pain assessment is obviously operator- and animal-model-dependent. Standard and validated international guidelines in the spirit of International Conference Harmonization guidelines for drug approvals would be extremely helpful for comparing all these delivery systems. The absence of standardized methods could explain why only a few formulations are the subject of ongoing clinical trials or on the market.

Inflammation, neurotoxicity and myotoxicity are of the greatest concern and seem to be related to high local concentrations of LAs. Although several studies in the literature reported the absence of adverse cellular and tissue responses, it seems premature to conclude on the safety of high local levels of LA. In addition, recent methods of stimulation based on light or ultrasound could interfere and undermine the bone healing process. Finally, local production of ROS must be tightly regulated because ROS will dramatically affect bone cell health and thus bone regeneration. All these concerns need to be extensively investigated in further studies to recommend the clinical use of these delivery systems for the entire population.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding this review.

Chapter 4: Methodology

4.1 Properties of materials

Mechanical properties of materials determine how they react to needle insertion. Two mechanical properties were measured in our study: Young's modulus and fracture toughness. In our study, Young's modulus was measured by a torsional rheometer, while fracture toughness was measured by a needle insertion model.

4.1.1 Rheometers

Rheometers are devices measuring the rheological properties of fluid or soft solid, such as elasticity and viscosity, by employing different rotation, frequency, and shear rate on the materials. Rheometers are also used to understand the complex behavior of polymers in the large and relatively unexplored field of non-linear viscoelasticity (*122*). Common rheometers include the sliding plate rheometer, torsional rheometer (cone-plate, parallel-plate), capillary rheometer, and extensional rheometer (*122*). Typical measurements include oscillatory time sweep, frequency sweep, and strain sweep (*123*).

Shear modulus is a measure of the elastic shear stiffness of a material and is calculated as the ratio of shear stress to shear strain. Young's modulus is a mechanical property that measures the stiffness in the normal direction of solid material and is calculated from the slope of tensile or compressive stress and axial strain in the linear elastic region. Young's modulus can also be inferred from the shear modulus, based on the assumptions that the tested material is isotropic, homogeneous, and incompressible. The torsional rheometer (DHR-2, TA Instruments, USA) used in this study measured the material's complex viscosity by a frequency sweep and its shear elastic (storage) modulus and viscous (loss) modulus by an amplitude sweep at 25 °C.

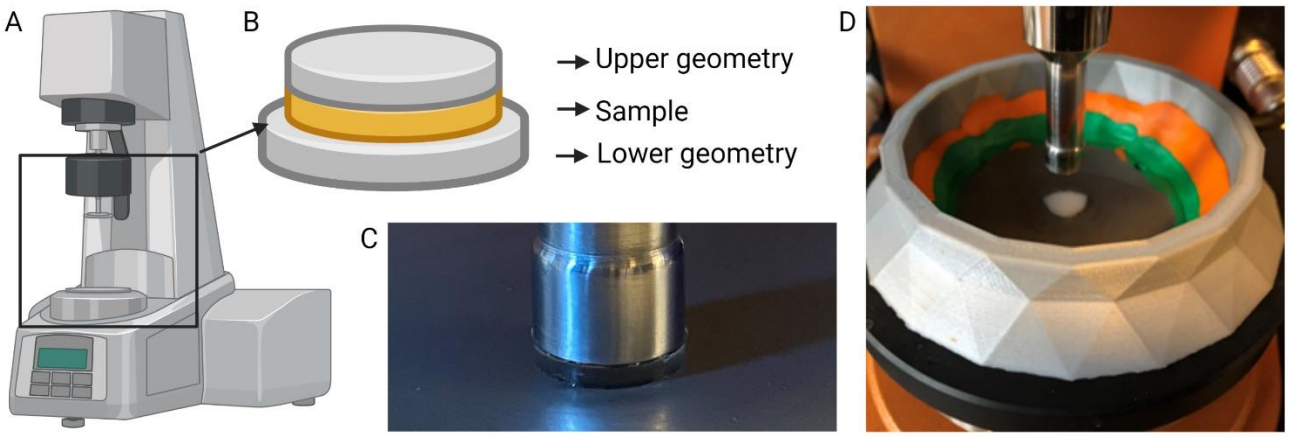

Figure 4.1 A diagram showing how the torsional rheometer works. (A) a Torsional Rheometer, (B) demonstration of sample placement. (C) Soft gel sample. (D) Soft tissue sample test in PBS bath. (A-B) were created with BioRender.com

4.1.2 Needle-insertion model for fracture toughness

Fracture toughness is the ability of a material to resist fracture (*67*). The ASTM standard recommends a shear test or a single-edged notch test to quantify the resistance of a material to failure by cracking, normally with specimens of coupon types (single-edge bending, compact tension, and disk-shaped compact tension coupon). However, these methods require specimens of large dimensions and take time to prepare. Two methods can determine the fracture toughness of real soft tissues without special preparation for the specimens: the scissor cutting test (*124*) or the needle insertion model (*125, 126*). The latter method was selected in this thesis due to its similarity to the standard needle injection procedure.

A typical needle insertion model for assessing fracture toughness includes a motorized linear transverse stepper (SPN7338, Velmex Inc, US), which controls the movement of needles, as well as a force transducer (GS0-500, transducer technique, USA), which records the force change during insertion (Figure 3.2). LabVIEW (LabView 2019, National Instruments, US) was used to program the needle movement and record the force data.

The needle is inserted into the phantom twice. The first insertion creates the deformation and penetration on the phantom, in which the friction, wedging, and work of fracture all contribute to the force. Subsequently, the second insertion is performed at the same location without creating the crack, requiring lower work and hence lower force (Fig 3.2 B). The difference of work between the two insertions is the work to create a crack in the material. The fracture toughness is thereby calculated by the work to create the crack divided by the area of needle travel (*126*).

$$\int_{\mu 2}^{\mu 1}(F_1 - F_2)\mathrm{d}\mu = J_{IC}\, a\, \mathrm{d}\mu \qquad Eq\ (1)$$

Where $\mu 1$ and $\mu 2$ are the beginning and end time points of the needle insertion, F_1 is the dynamic force during the first insertion, F_2 is the dynamic force during the second insertion, a is the cross-sessional area of the needle, and $\mathrm{d}\mu$ is the dynamic change of needle position. The fracture toughness J_{IC} could be calculated from the slope of the Eq(1) (*127*).

The measurement of fracture toughness is also affected by the diameter and shape of the needle as well as the indentation rate during needle insertion (*125*, *126*). Therefore, proper and consistent needle diameter, shape and indentation rate should be selected. In our study, we used 5mm/s indentation rate, 25-gauge needle with bevel tip for testing porcine oral mucosa, muscles, liver, and all the phantom materials.

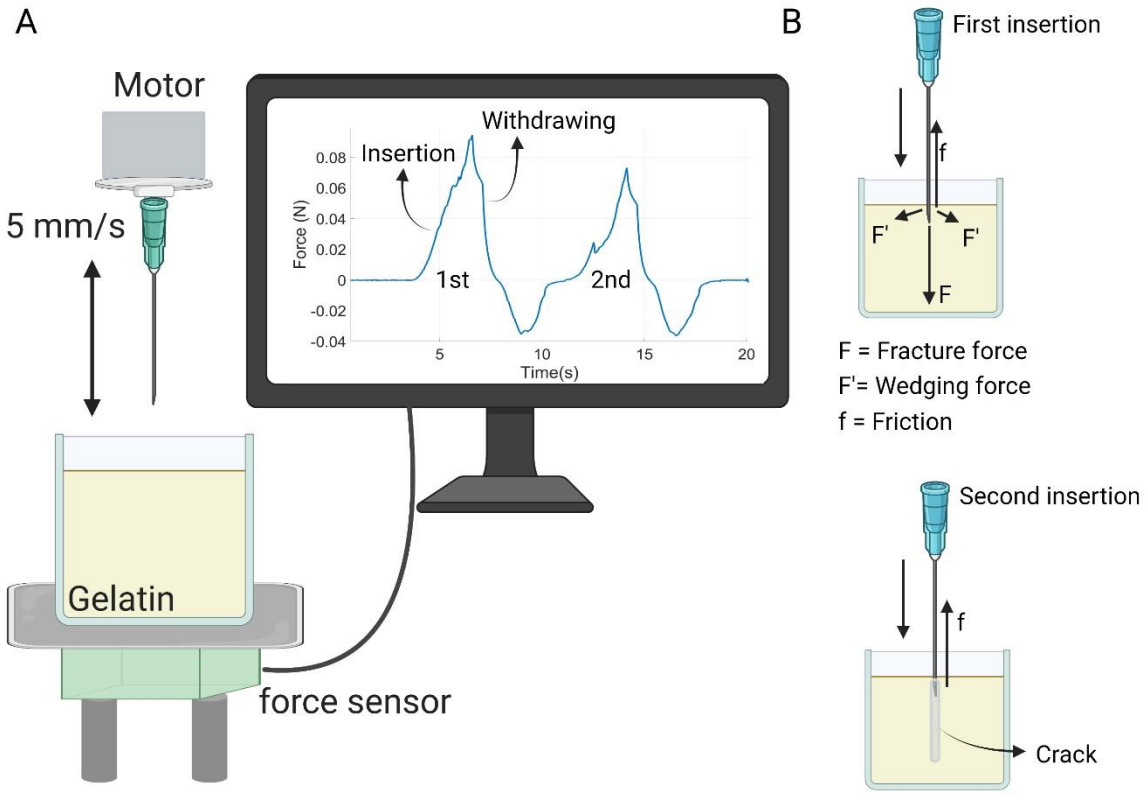

Figure 4.2 (A) The experimental setup of the needle insertion model for fracture toughness. (B) The force and friction during needle insertion. Figures were created with BioRender.

4.1.3 The force transducer

The force transducer is the most important component in the needle-insertion model for fracture toughness, as it records the force change during injections. A force transducer is a sensor that converts input mechanical load, such as weight, tension, compression, and pressure, into electrical output signals. There are several types of sensors based on size, geometry, and capacity. The most common force sensors are the piezoelectric crystal force transducer and the strain gauges force transducer (*128*).

The piezoelectric type has a metal plate bonded onto the surface of the crystal, which is made of quartz or lead zirconate titanate. The atoms inside the crystal could be displaced when

force is applied. This displacement can result in a net charge on the opposite face of the crystal, which can then be measured electrically (*128*).

A strain gauge is a metal or semiconductor whose resistance changes when it is deformed. The deformation is usually taken to be a measurement of strain, and hence force, applied to a structure (*128*). The piezoelectric force transducers work accurately for rapidly changing forces, but the accuracy decreases when the force is constant or changes with low frequency (*128*).

Moreover, the amplitude force during jet injection in our study was too small for the piezoelectric sensor to measure accurately. As the strain gauge force transducer is relatively more sensitive and accurate for constant force or low value force, without transient response, it was selected for our study (GS0-500, transducer technique, USA).

4.2 Investigation of NFLJI

4.2.1 High-speed imaging

High-speed imaging (HSI) has been widely used for investigating high-speed liquid jets (*1*) (*129*). It captures the injection procedure at 5000-20000 frames per second (fps), which allows the researcher to analyze the needle-free liquid jet injection in detail. The high-speed camera in our study has a frame rate of up to 1000 fps (Fastcam MC2, Photron, Japan).

4.2.2 Data acquisition and processing

During each needle-free injection, the trajectory of the liquid jet inside the phantom material and the force change during the jet penetration were respectively recorded by a high-speed camera and a force transducer. The NFLJI system, high-speed camera, and force transducer were controlled by LabVIEW (LabView 2019, National Instruments, US) (Figure 3.3) to start simultaneously. The image processing was done using motion tracking software (PFA, Photron, Japan), and the force transducer data was processed with MATLAB (The MathWorks, Inc, USA).

The following variables were extracted from high-speed video or sensor output and then analyzed: the penetration depth of jet as the function of time, the duration of injection, the dynamic force change as the function of time, and the mean and maximum force during the injection. All the variables were used to calculate jet velocity, jet impulse, and total work during the injection.

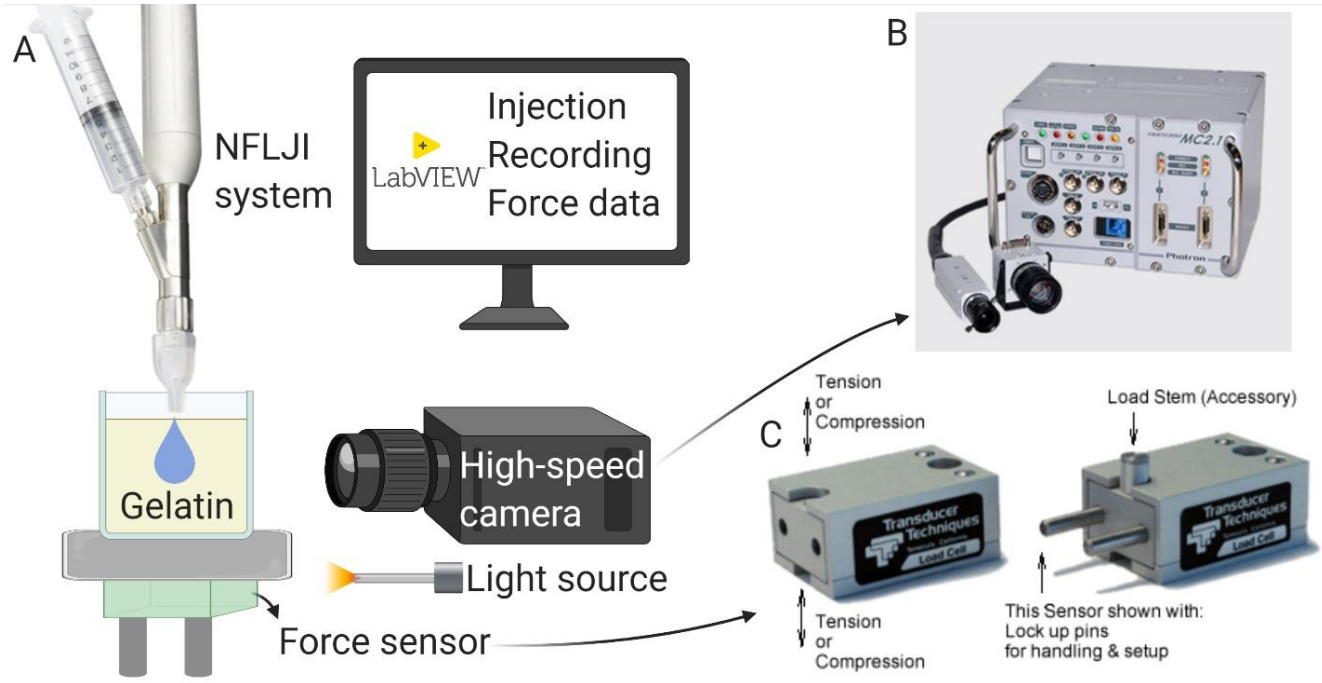

Figure 4.3 (A) The experimental set-up in our study, (B) the high-speed camera (Photron Fastcam MC 2.1) and (C) the force transducer (GS0-500, transducer technique, USA). Figure (A) was created with BioRender.

4.3 Cadaveric studies

Cadaveric research is widely used to investigate the effect of local anesthesia techniques. Generally, the operators first perform the anesthesia technique on cadavers by injecting 0.2% methylene blue (*130*), and then dissect the injection site to check if the anesthesia was successful. The definition of successful anesthesia on a cadaver mainly depends on whether the nerves are adequately stained (*131*) and whether the nerves are at the center of the stained area (*132*). However, this standard evaluation based on straining patterns is subjective. To address this limitation, our study employed four blinded assessors who scored the digital picture and agreed with the conclusion.

4.4 Dental anesthesia techniques

Common dental anesthesia techniques include infiltration anesthesia, mental incisive nerve block (MINB), and inferior alveolar nerve block (IANB) (*12*). Infiltration anesthesia simply blocks the pain signal of the individual tooth and the surrounding soft tissue by depositing anesthetics near the tooth region (*12*). There are different NFLJI techniques for infiltration anesthesia depend on the impinge angle (Figure 3.4). Our study showed that perpendicular and oblique NFLJI had different clinical outcome.

The nerve blocks inhibit the pain signal of a large area by depositing anesthetics near the main nerve bundles. For example, the MINB is injected from the mucobuccal fold of the mandibular vestibule at the lower premolar region to ensure that the anesthetics surround the mental foramen, thereby blocking the mental nerve.

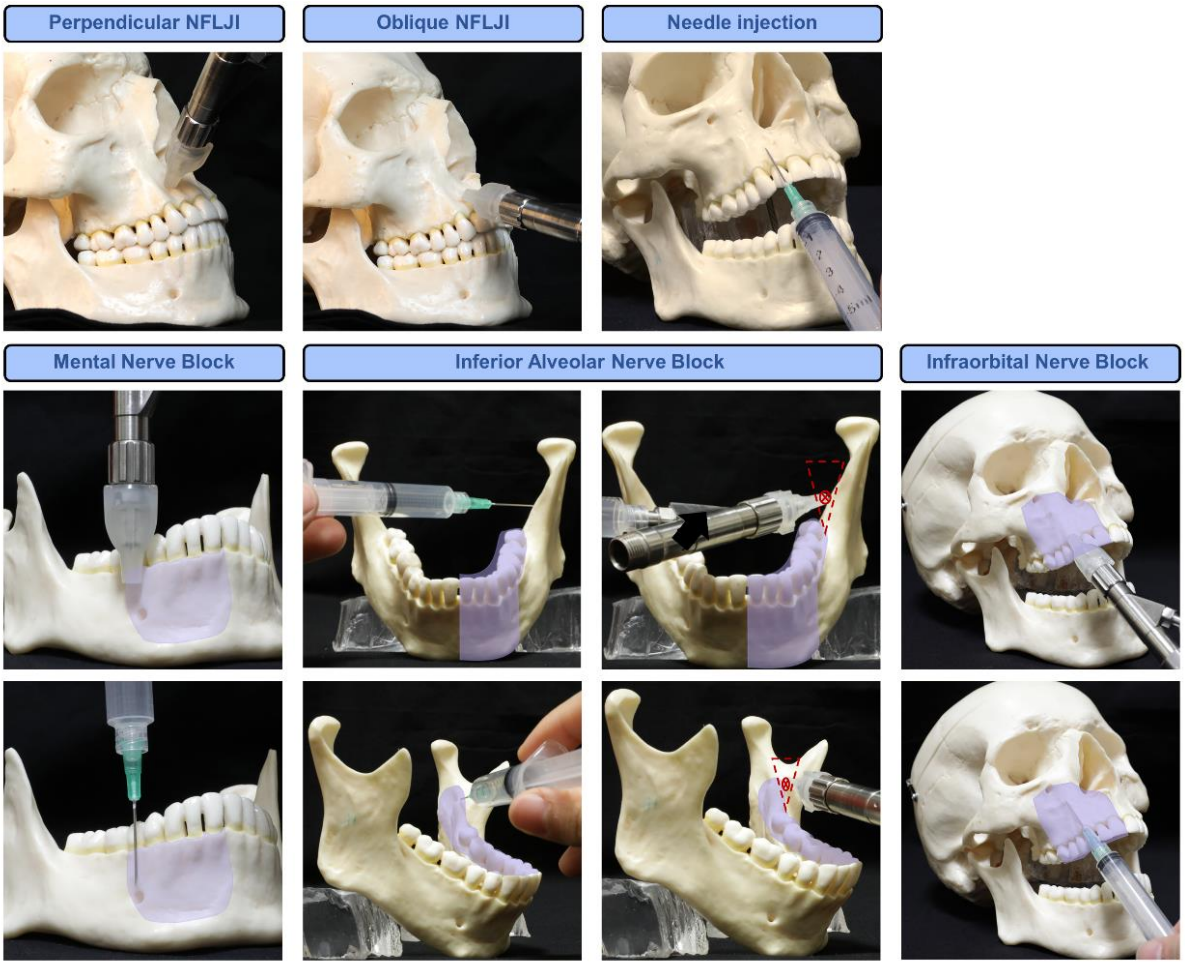

Figure 4.4 the demonstration of common dental anesthesia technique on skulls.

4.5 Clinical Trials

Randomized control trials (RCT), when appropriately designed, conducted, and reported, represent the gold standard in evaluating healthcare interventions (*133*). An RCT is a study in which participants are allocated randomly into the control and experimental groups to receive different interventions.

A pilot and feasibility trial is a study conducted in advance of future definitive RCT, in which a future RCT is conducted on a smaller scale (*134*). Although much of the information to be reported in these trials is similar to that of RCTs assessing effectiveness and efficacy, there are some critical differences in the type of information and the appropriate interpretation of standard CONSORT reporting items. The aims of the pilot and feasibility studies differ from those of RCTs. The focus is on assessing the feasibility of further development rather than assessing effectiveness or efficacy. Moreover, the limited sample size of pilot and feasibility studies cannot provide reasonable statistical power to conclude the effectiveness or efficacy.

A non-inferiority randomized trial seeks to determine whether a new treatment is not worse than a reference treatment by more than an acceptable amount (*135*). Because proof of exact equivalence is impossible, a pre-stated margin of non-inferiority for the treatment effect in a primary patient outcome is defined (*135*). Noninferiority of the new treatment with respect to the reference treatment is of interest on the premise that the new treatment has some other advantage, such as greater availability, reduced cost, less invasiveness (*136, 137*), fewer adverse effects (harms) (*138*), or greater ease of administration (*139*).

All the RCTs with the design mentioned above should be approved by the institution's research ethics board and registered online (https://clinicaltrials.gov/). They should further be designed and reported according to the CONSORT guidelines (*133-135*)5

Chapter 5 Needle-free injection: dental infiltration anesthesia

5.1 Preface

In this chapter, we investigated the high-speed liquid jet for infiltration anesthesia, targeting the superficial nerve end at the dentoalveolar region, characterized with a thin layer of mucosa supported by rigid bone. The fluid dynamics of NFLJI in the dentoalveolar region were investigated using soft tissue phantoms supported by rigid glass. An optimized NFLJI for infiltration anesthesia was developed and validated on cadavers, then assessed in two pilot randomized control trials (RCT) for its safety and feasibility.

This chapter(*140*) has been published in the "international journal of pharmaceutics".

Gao Q, Noel G, Der Khatchadourian Z, Taqi D, Abusamak M, Henley A, Menassa K, Velly A, Emami E, Mongeau L, Tamimi F. Needle-free Injection: Dental Infiltration Anesthesia. International Journal of Pharmaceutics. 2021 Jun 1:120765.

https://doi.org/10.1016/j.ijpharm.2021.120765

Needle-free Injection: Dental Infiltration Anesthesia

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Abstract word count:277. Total word count:4047

No. of figures: 4. No. of Tables: 3. Appendix table: 2

No. of References: 54

This study has been registered online with the title needle-free dental anesthesia (NCT04493528), https://clinicaltrials.gov/ct2/show/NCT04493528.

Highlight:

- The needle-free liquid jet injection outcomes are correlated with the impact angle.
- The oblique needle-free injection is relatively safe with fewer complications.
- It is feasible to conduct a non-inferiority randomized controlled trial.

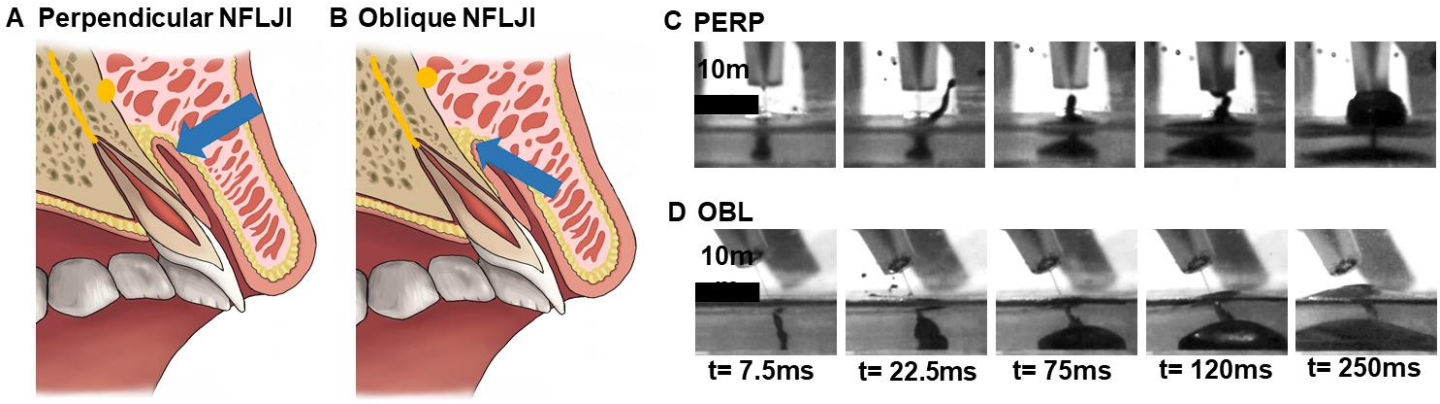


Figure 5.1 Graphic abstract

5.2 Abstract

This study aimed to develop an optimal Needle-Free Liquid Jet Injection (NFLJI) technique for dental infiltration anesthesia and evaluate its clinical safety and feasibility. The fluid dynamics of NFLJI in the dentoalveolar region were investigated using soft tissue phantoms supported by rigid glass. NFLJIs were performed at different incident angles and recorded by a high-speed camera. Accordingly, an optimal NFLJI for infiltration anesthesia was developed and validated on cadavers, then assessed in two pilot randomized control trials (RCT): one trial for validating the safety of optimal technique, the other for evaluating its feasibility and safety. High-speed videos showed that perpendicular NFLJIs induced significantly more regurgitation than oblique NFLJIs, which was confirmed in cadavers. Clinical trials revealed that perpendicular NFLJIs induced a high risk of bleeding (83.3%) and laceration (83.3%), whereas oblique NFLJIs induced a low risk of bleeding (33.3 %) and laceration (16.7%). Moreover, the preliminary success rates of oblique NFLJIs and needle injections were both 83.3%. The recruitment took 3-5 weeks with a rate of 100%. Oblique NFLJIs could be a promising approach for dental infiltration anesthesia, causing minimal drug regurgitation with a relatively low risk of complication. The pilot RCTs confirmed the feasibility for conducting a non-inferiority RCT.

Keywords: Local Drug Delivery; Infiltration Anesthesia; Jet Injections; Pilot Studies; Feasibility studies; Randomized Clinical Trial; Complications.

Abbreviations: NFLJI, Needle-Free Liquid Jet Injection; RCT, Randomized Controlled Trial.

5.3 Introduction

Needle phobia, due to injection pain and anxiety, is reported by 10-20% of dental patients (*4, 5*). It may cause avoidance of necessary dental treatments (*3, 5*). Injection pain is caused by

needle injury, distension and inflammation due to percolation of the injected fluid, and mucosa irritation in response to the anesthetic (*13*). Furthermore, needle injection can cause complications such as needlestick injuries (*141*), hematoma, needle fracture (*6*) and the transmission of diseases such as HIV or HBV (*1, 142*).

In order to reduce the above-mentioned problems and improve patients' experience, needle-free injection systems have been studied for the administration of anesthesia, for example powder jets (*143*) and liquid jets (*1*). Different power supplies have been used, including mechanical springs, laser light or pressurized gas (*49*). Needle-free liquid jet injection (NFLJI) systems have been used in dermatology (*7*) as well as for the delivery of vaccines (*8, 9*) , insulin (*10*), and growth hormones (*11*). These systems employ high-velocity jets to deliver liquids across the skin into the subcutaneous or intramuscular region (*1*). NFLJI systems are considered a cost-effective alternative to needle injection because they can accommodate existing anesthetic commercial formulas designed for needle injection (*1*). They also have the potential to reduce pain during injection (*52, 144*).

Despite their potential advantages, NFLJI systems are rarely used in dental practice, mainly due to their inconsistent efficacy (Appendix Table 1) and unpredictable complications, such as bleeding and discomfort, unpleasant taste, and pain (*16, 17*). For local infiltration anesthesia, the anesthetic must be delivered locally to block the small nerve terminals of the tooth apices (*12*). The dentoalveolar region offers a unique set of challenging conditions for NFLJI due to the presence of hard bones underlying the thin layer of soft tissues. In this context, we hypothesized that the hard tissue in the dentoalveolar region could affect NFLJI fluid dynamics, which implies that the angulation of NFLJI could affect its outcomes.

Accordingly, this study investigated the fluid dynamics of NFLJI as a function of injection angle in the dentoalveolar region, aimed to develop an optimal NFLJI technique for infiltration anesthesia, and evaluate its clinical safety and feasibility.

5.4 Materials and Methods

The NFLJI system in this study has a nozzle diameter of 120 μm, adjustable injection pressure supply of 413- 1400 psi, and adjustable volume of 0.1ml- 1.8ml (Medical International Technologies Inc, Montreal, Canada).

5.4.1 NFLJ fluid dynamics on soft tissue phantoms

To simulate the structure of the dentoalveolar region, which is a thin layer of soft tissue supported by rigid bone, a design of 3-20 mm soft tissue phantom (10 wt.% gelatin, Sigma-Aldrich, US) (Cronin and Falzon 2011) supported by a glass plate (Fig 2A) was used for the injection test. Methylene blue (0.2 %) was used as the injection agent to visualize the injection outcome (*130*) and was injected (620kPa, 0.3ml) at different incidence angles from 90° to 45° (Figure 2 A) into the soft tissue phantom.

A High-Speed Imaging system (FASTCAM MC2.1, Photron, Japan) was used to capture the NFLJI at 6000 fps and obtain the velocity profile, the jet penetration, and regurgitation in the dentoalveolar phantom. The puncture wound size and dispersion area were photographed as digital images and measured by software (ImageJ, USA) (*145*)

Regurgitated fluid was collected using a pipette and transferred to a weighing dish, then weighted using an analytical balance (Quintix64, 60 g × 0.1 mg, Sartorius, German). The percentage of regurgitation was calculated as the regurgitated fluid mass divided by the total fluid mass.

5.4.2 Preclinical investigation of NFLJI fluid dynamic in the dentoalveolar region

Two cadavers (Figure 3 B, C, F, G) in Thiel preservation (*146*) were used to compare between perpendicular and oblique NFLJIs. The cadaver study was approved by the McGill Ethics Review Board (A09-M36-18A). On each cadaver, one perpendicular or oblique NFLJI (0.3ml at 620 kPa) was randomly performed by an experienced dentist in the left or right upper lateral incisor region, followed by dissections performed by an experienced anatomist. The injection regions were photographed immediately after injections and dissections.

An injection volume of 0.3 mL was selected for the laboratory and cadaveric studies. This volume is sufficient for investigating the NFLJIs dispersion in gelatin(*129*) and assessing how NFLJIs can target the desired region on cadavers(*65*).

5.4.3 Clinical validation of optimal NFLJI technique

To evaluate the safety and feasibility of oblique and perpendicular NFLJIs, a pilot split-mouth cross-over randomized controlled trial was conducted and reported according to the CONSORT guideline for pilot and feasibility trials (*147*) (Figure1A). This study was approved by the McGill Research Ethics Board (A09-M36-18A), registered online (NCT04493528), and executed at the McGill Student and Staff Dental clinic during Aug 01-Sep13, 2019.

Six participants were recruited. It was impossible to formally calculate a standard sample size due to inconsistencies in previously reported data. The eligible participants had to be 18~35 years old and fluent in English. Participants with chronic pain, systematic disease, or root canal treatment at the upper lateral incisor were excluded. Data collection stopped after reaching six participants.

After signing the consent form, all participants received one perpendicular NFLJI (Figure3E) and one oblique NFLJI (Figure3A) at either the left or right upper lateral incisor region. The sequence, location, and interventions were randomly assigned as matched sets in

sealed envelopes (*148*) by the research coordinator. All injections were performed by one experienced dentist using 1 mL 2 % Lidocaine and 1:100,000 epinephrine. This volume was selected to provide reasonable anesthesia success rate according to previous studies (*15*) (*31*) and guidelines (*12*). The NFLJI system delivery pressure was 900 kPa. Participants waited for 60 minutes between two interventions.

For the NFLJI technique, the operator followed a "locate-place-orient-stabilize -deliver" procedure. The operator first located the injection site at the mucobuccal fold above the upper lateral incisor. After drying the mucosa, the operator gently placed the tip on the mucosa. The barrel axis was oriented obliquely (45°) (Figure3A) or perpendicularly (Figure 3E) to the bone surface. To stabilize the device, the operator squeezed the armpit to fix the upper arm, used three fingers to hold the device, while using the little finger to support the chin. To deliver the anesthetic, the operator pressed the trigger firmly for 3 seconds, then released the trigger.

The primary outcomes of this pilot RCT were the feasibility and safety, including the recruitment time and rate, participant's concerns, withdrawal rate, problems in methods, and complications. Secondary outcomes were the preliminary success rate and duration of anesthesia, pain numerical rating score, and taste score. The outcomes were measured in chronological order as follows:

a. The initiation and termination of soft tissue anesthesia were recorded upon participants' reports, then confirmed by the pinch test (*12*).

b. The dental pulp anesthesia was first reported by participants, then confirmed by electric pulp testing (EPT) on the tooth showing no response to maximal electric output (80/80) (*12*).

c. Pain levels before and during injection were measured using the numeric rating scale (NRS) for pain (*149*).

d. Taste scores before and after injection were measured using a 9-point hedonic scale (*150*).

e. Any sign of complications was recorded after the procedure and followed for one week, including but not limited to bleeding, hematoma, laceration, nausea, tachycardia, and nerve paralysis.

f. Participants' feelings, concerns, and complaints were collected at the end of the questionnaire.

g. The recruitment time, recruitment rate, and withdrawal rate were calculated after the trial. Participants were monitored in the clinic until the anesthesia ended and were followed for one week. Topical anesthetics were not used. Only the data assessor was blinded since it was not possible to blind the operator and participants.

5.4.4 Feasibility of Comparison between oblique NFLJIs and needle injections

A second pilot RCT with another six participants was conducted to evaluate the feasibility of conducting a formal RCT to compare oblique NFLJIs and needle injections for infiltration anesthesia. The study design, inclusion and exclusion criteria, primary and secondary outcomes, blinding and randomization process, and dosage of anesthetic were the same as described above (Figure 1B). This clinical trial was conducted over Jan 01-Mar 05, 2020, at the McGill Dental Clinic.

An experienced dentist randomly delivered one oblique NFLJI (Figure 4E) or one needle injection (Figure 4A) in the upper left or right lateral incisor region for each participant. Needle injections were performed using #30 needles injecting 1 ml of 2 % Lidocaine with 1:100,000 epinephrine at 2 mL/min (*151, 152*). Oblique NFLJIs were performed as mentioned above. Four refinements were made: the NFLJI pressure was reduced from 900 kPa to 482 kPa to minimize discomfort. The numeric rating scale (NRS) was replaced by a visual analog scale (VAS) for a more sensitive pain assessment (*153*). Additionally, an anxiety VAS was added to evaluate

participants' anxiety levels before and during the anesthesia procedure(*154*). Participants were asked about their preferences at the end of the appointment.

Before the clinical trial, oblique NFLJIs and needle injections were tested in soft tissue phantoms and four cadavers to compare the dispersion effects (Figure 4 B, C, F, G) using the same method as mentioned above.

5.4.5 Statistical Analysis

Descriptive analysis was performed using SPSS (Version 21.0, IBM, SPSS statistics) and Prism8 (GraphPad Software, San Diego, California, USA). Categorical variables, such as the success of soft tissue and dental pulp anesthesia as well as cases of complications, were reported as count and percentage. Continuous variables, such as duration of anesthesia, pain and anxiety score, and taste score, were reported as median and interquartile range (IQR: 25^{th} percentile – 75^{th} percentile).

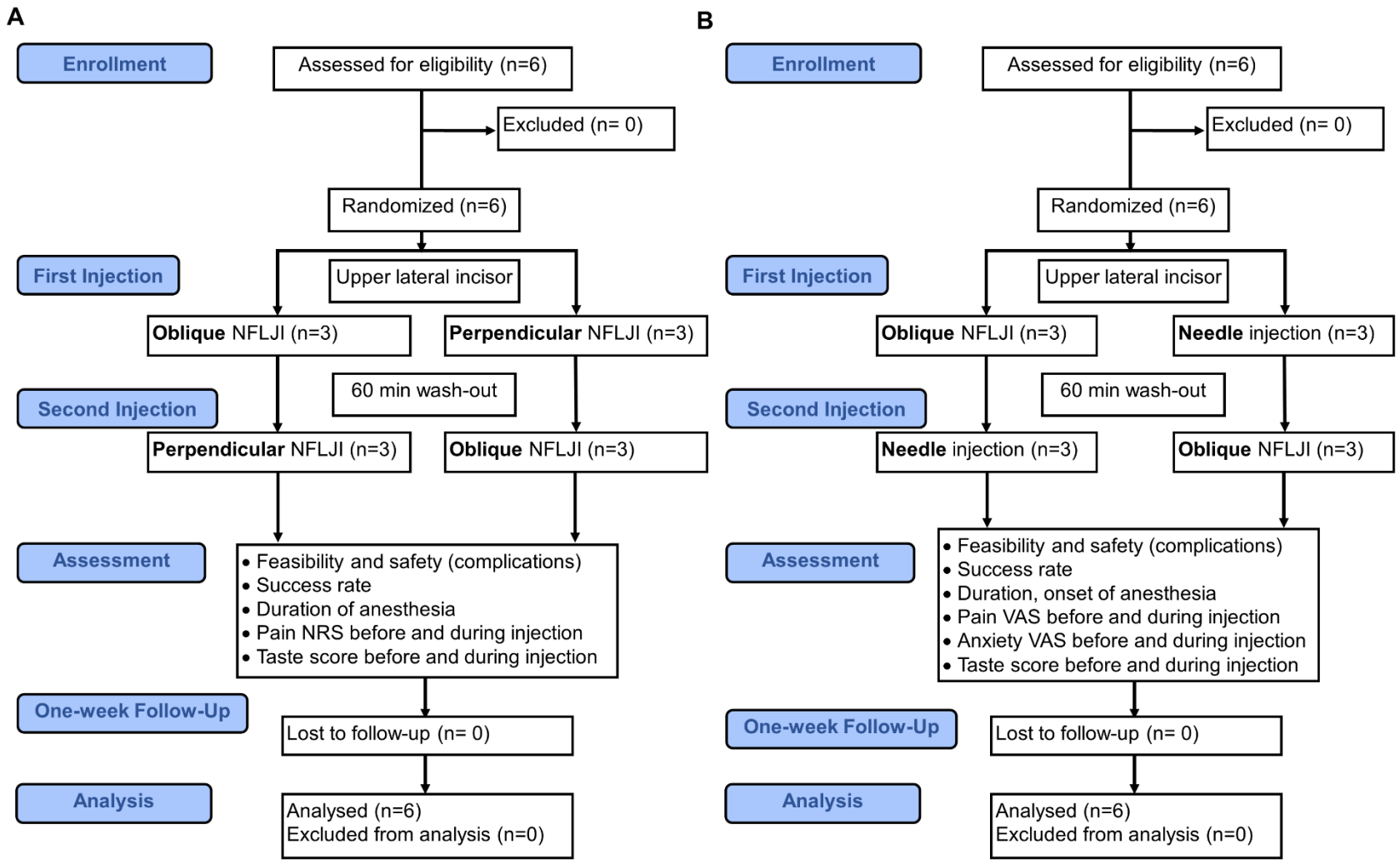

A
Enrollment
Assessed for eligibility (n=6)
Excluded (n= 0)
Randomized (n=6)
First Injection
Upper lateral incisor
Oblique NFLJI (n=3)
Perpendicular NFLJI (n=3)
60 min wash-out
Second Injection
Perpendicular NFLJI (n=3)
Oblique NFLJI (n=3)
Assessment
• Feasibility and safety (complications)
• Success rate
• Duration of anesthesia
• Pain NRS before and during injection
• Taste score before and during injection
One-week Follow-Up
Lost to follow-up (n= 0)
Analysis
Analysed (n=6)
Excluded from analysis (n=0)
B
Enrollment
Assessed for eligibility (n=6)
Excluded (n= 0)
Randomized (n=6)
First Injection
Upper lateral incisor
Oblique NFLJI (n=3)
Needle injection (n=3)
60 min wash-out
Second Injection
Needle injection (n=3)
Oblique NFLJI (n=3)
Assessment
• Feasibility and safety (complications)
• Success rate
• Duration, onset of anesthesia
• Pain VAS before and during injection
• Anxiety VAS before and during injection
• Taste score before and during injection
One-week Follow-Up
Lost to follow-up (n= 0)
Analysis
Analysed (n=6)
Excluded from analysis (n=0)

Figure 5.2. **CONSORT Flowchart for two pilot clinical trials.** (A) The first trial aimed to validate oblique and perpendicular NFLJIs, and to evaluate their safety and feasibility; (B) The second trial aimed to evaluate the safety and feasibility of conducting a larger RCT to compare oblique NFLJIs and needle injections for infiltration anesthesia.

5.5 Results

5.5.1 NFLJI regurgitates when injected into soft tissue supported by hard tissue

Perpendicular NFLJI created significant fluid regurgitation when injected into soft tissue supported by hard tissue, as revealed in high-speed imaging (Figure 2D), and this was regardless of the thickness of the overlying soft tissue phantom (Figure 2F). After the liquid jet penetrated the soft tissue phantom perpendicularly, the entering stream and its backflow created a flow recirculation region between the rigid surface and the overlying soft tissue phantom. This backflow reduced the vertical momentum and stagnation pressure and increased fluid regurgitation towards the initial puncture site (Figure2G).

5.5.2 Oblique injections reduce NFLJI regurgitation and produce similar dispersion as needle

To reduce the NFLJI regurgitation described above, we investigated the effect of incidence angle. The flow momentum is a vector quantity equal to the product of density and velocity. Oblique angle divides it into the sum of two orthogonal components: one is still perpendicular to the surface; another one is parallel to the surface. The perpendicular component and its backflow are hence reduced, whereas the parallel component produces no backflow.

Oblique NFLJIs were observed to create significantly less regurgitation, smaller puncture wounds, and larger dispersion area than perpendicular NFLJIs (Figure2B), regardless of the incidence angle (Figure 2C) or the thickness of the soft tissue phantom (Figure 2F). High-speed imaging (Figure 2D&E) revealed the detailed steps during injection and how oblique NFLJI created a different flow momentum than perpendicular NFLJI (Figure 2G). The cadaveric study results agreed with *in vitro* observations and confirmed that oblique NFLJI produced less fluid regurgitation than the perpendicular one (Figure3 B, C, F, G).

Compared to needle injection, oblique NFLJI (Figure 4F&B) created a similar dispersion profile. On cadavers, the dispersion profiles showed that both oblique NFLJI and needle injection infiltrated a similar area underneath the mucosa (Figure 4C, G).

In addition, NFLJIs, using a supply pressure of 413-900 kPa and an injection volume of 1 mL, showed a mean exiting velocity of 72.3 (SD 5.9) m/s, which is measured frame by frame using high-speed camera recordings. This jet exiting velocity gave a mean driving pressure of 2.6 MPa according to the Bernouli's equation (*155*). The jet energy when it exits the orifice is different from the energy when gas pressure impacts the injector piston due to the friction, and the different mass and area between piston and liquid.

5.5.3 Clinical feasibility and safety of oblique and perpendicular NFLJIs

To evaluate the safety and feasibility of oblique and perpendicular NFLJIs, a pilot cross-over split-mouth randomized clinical trial was conducted. A total of 6 participants (3 males, 3 females) were recruited with median age of 26.5 years old (IQR 25-30) (Table 1).

While the most significant barrier was recruitment, the recruitment rate increased to 100% after we posted advertisements on social media. The recruitment took five weeks and no participants reported concerns or withdrew from the study. Both oblique and perpendicular NFLJIs procedures were easily performed intraorally.

Both oblique and perpendicular NFLJIs achieved preliminary success rates of 100% for soft tissue anesthesia and 66.7% for dental pulp anesthesia (Figure 3I, 3J), and the anesthesia lasted for 189 (183-221) min and 209 (158-231) min respectively (Figure 3K, Table 1).

In terms of side effects, participants who received oblique NFLJIs presented median (IQR) pain NRSs of 3(1.6-6.4) cm and taste scores of 5.0 (3.8-5.5), while the perpendicular

NFLJI group showed pain NRSs of 3.5 (1.0-6.3) cm and taste scores of 4.5(4-5.75) (Figure 3L, M).

Perpendicular NFLJIs, however, showed a high risk of complications for bleeding [Figure 3N, 5 (83.3%)] and laceration [Figure 3O, D, 5 (83.3%)], whereas oblique NFLJIs had a low risk of complication for bleeding [Figure 3N, 2(33.3%) and lacerations [Figure 3O, D, 1(16.7%)]. The bleeding ceased within 3 min after injection, and the laceration recovered within one week.

Although both techniques achieved successful infiltration anesthesia, oblique NFLJI was considered a relatively safe approach due to its low risk of complication.

Table 5.1 Demographic and clinical outcomes for the first pilot randomized clinical trial evaluating the safety and feasibility of oblique and perpendicular NFLJIs.

Demographic Outcomes		
Age (years), median (IQR)	26.5(25-30)	
Gender (F), n (%)	3(50%)	
Clinical Outcomes	**Oblique NFLJI (n=6)**	**Perpendicular NFLJI (n=6)**
Soft tissue anesthesia success rate, n (%)	6 (100%)	6 (100%)
Dental pulp anesthesia success rate, n (%)	4 (66.7%)	4 (66.7%)
Duration of anesthesia (min), median (IQR)	189 (183-221)	209 (157-231)
Bleeding, n (%)	2 (33.3%)	5 (83.3%)
Laceration, n (%)	1 (16.7%)	5 (83.3%)
Pain NRS difference (0-10), median (IQR)	3.0 (1.6-6.4)	3.5 (1.0-6.3)
Taste preference score (1-9), median (IQR)	5.0 (3.8-5.5)	4.5 (4-5.75)

5.5.4 The feasibility of comparing oblique NFLJIs and needle injections

To evaluate the feasibility for conducting a cross-over split-mouth randomized clinical trial comparing oblique NFLJI and needle injection, a total of 6 participants were recruited. Demographically, these participants included 2 males and 4 females with a median age of 21.5 (IQR 19-22) years old.

Regarding the effects of anesthesia, both oblique NFLJI and needle infiltration achieved preliminary success rates of 100% for soft tissue anesthesia and 83.3% for dental pulp anesthesia (Figure 4I, J; Table 2). The respective anesthesia duration were 208 (183-222) minutes and 193

(109-211) minutes (Figure 4K). The oblique NLJFI group showed the time to initial anesthesia of 1.1(0.9-1.5) minutes, time to onset of 3.7 (3.2-5.1) minutes, pain VAS difference of 3.0(0.5-4.3) cm before and during injection, anxiety VAS difference of 2.5(1.1-33.8) cm, taste score difference of 4.5 (3.3-5.0). The needle injection group showed the time to initial anesthesia of 0.8 (0.5-1.1) minutes, time to onset of 3.4 (2.7-6.3) minutes, pain VAS difference of 2.5 (1.4-4.1) cm, anxiety VAS difference of -0.2 (-0.4-1.7) cm, and taste score difference of 5.0 (5.0-5.0) (Figure 4 L- N, Table 2).

In terms of complications, the oblique NFLJI group had 2 (33.3%) cases of bleeding and 3 (50%) cases of laceration, whereas the needle injection group had 0 (0%) cases of bleeding and 0 (%) cases of laceration (Figure 4O-P; Table 2). Two participants moved their heads when receiving the injection. The real laceration rate for oblique NFLJI would be 17% if these two cases were removed. The bleeding stopped within 3 minutes following the injections, and lacerations healed within one week.

The recruitment took three weeks. All participants accepted and showed up after signing the consent form. No participants raised any concerns about the novel needle-free anesthesia. No participants withdrew from the study. Regarding the preference, 1(16.7%) participant preferred needle-free anesthesia, 2(33.3%) preferred needle injections, and 3(50%) accepted both methods.

Table 5.2 Demographic and clinical outcomes for the second pilot randomized controlled trial for oblique NFLJI and needle injection.

Demographic Outcomes	
Age (years), median (IQR)	21.5 (19-22)
Gender (F), n (%)	2 (33%)

Clinical Outcomes	Oblique NFLJI (n=6)	Needle injection (n=6)
Soft tissue anesthesia success rate, n (%)	6 (100%)	6 (100%)
Dental pulp anesthesia success rate, n (%)	5 (83.3%)	5 (83.3%)
Duration of anesthesia(min), median (IQR)	208 (183-222)	193 (109-211)
Time to initial anesthesia(min), median (IQR)	1.1 (0.9-1.5)	0.8 (0.5-1.1)
Time to onset(min), median (IQR)	3.7 (3.2-5.1)	3.4 (2.7-6.3)
Bleeding, n (%)	2 (33.3%)	0 (0%)
Laceration, n (%)	3 (50%)	0 (0%)
Pain VAS difference, median (IQR)	3.0 (0.5-4.3)	2.5 (1.4-4.1)
Anxiety VAS difference, median (IQR)	2.5 (1.1-3.8)	-0.2 (-0.4-1.7)
Taste preference score, median (IQR)	4.5 (3.3-5.0)	5.0 (5.0-5.0)

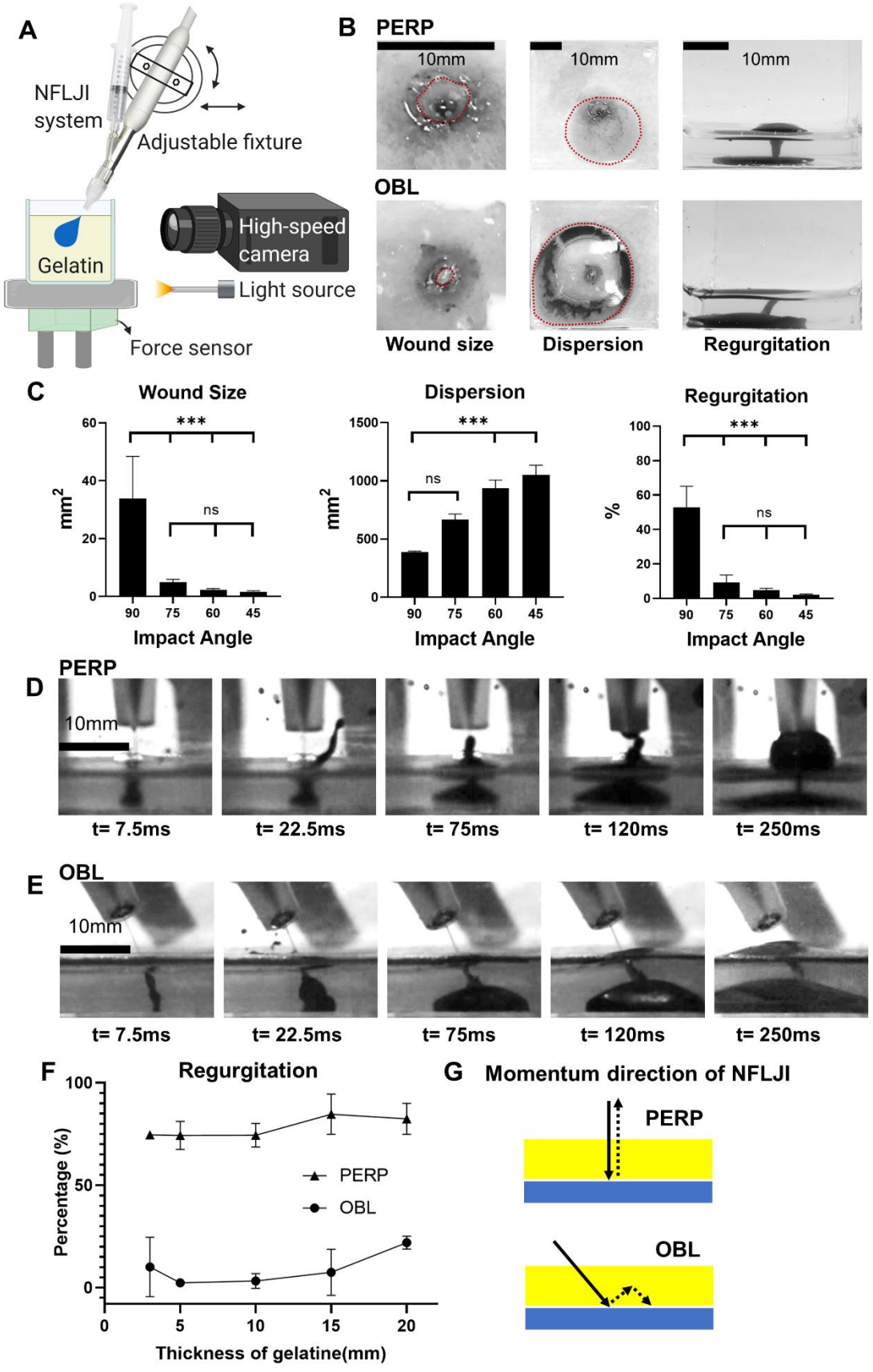
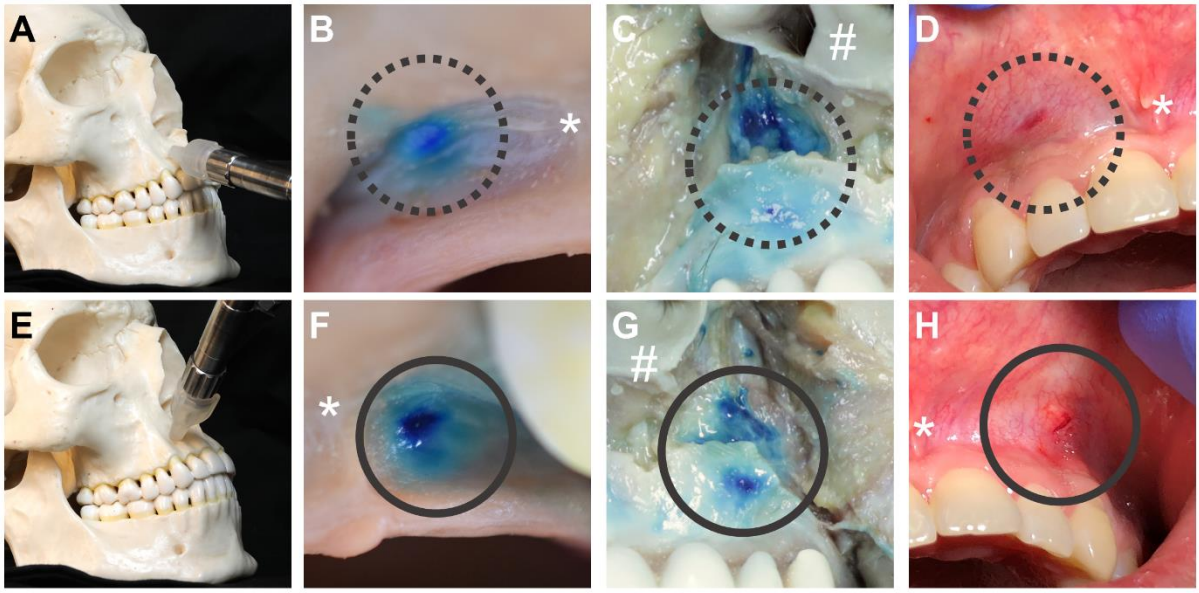

A
NFLJI system
Adjustable fixture
Gelatin
High-speed camera
Light source
Force sensor
B
PERP
10mm
OBL
Wound size
Dispersion
Regurgitation
C
Wound Size
Dispersion
Regurgitation
mm2
%
***
ns
Impact Angle
90
75
60
45
D
PERP
10mm
t= 7.5ms
t= 22.5ms
t= 75ms
t= 120ms
t= 250ms
E
OBL
F
Regurgitation
Percentage (%)
PERP
OBL
Thickness of gelatine(mm)
G
Momentum direction of NFLJI
PERP
OBL

Figure 5.3. In vitro experiment of Needle-free liquid jet injection (NFLJI) using different impact angles. (A) Experimental setup. (B) The puncture wound size, dispersion area, and regurgitation effect for perpendicular injection compared to oblique injection at 60°. (C) Comparison between NFLJI with different incidence angles (90°,75°,60°,45°) on 5 mm soft tissue phantom. High-speed camera recording showed that (D) Perpendicular NFLJI produced a more significant amount of liquid regurgitation than (E) oblique NFLJI. (F) quantitative comparison of regurgitation between perpendicular and oblique NFLJI at the different thickness of phantom. (G) Theoretical model: In perpendicular NFLJI, the injection stream and the subsequent vertical backflow collide, which reduces the momentum of entering stream and stagnation pressure. In oblique NFLJI, the transverse momentum component was preserved even though the vertical momentum was reduced, resulting in horizontal substantial fluid dispersion between the soft tissue and bone surface and causing less fluid regurgitation.


A
B
C
D
E
F
G
H
#
*
Oblique NFLJI
Perpendicular NFLJI

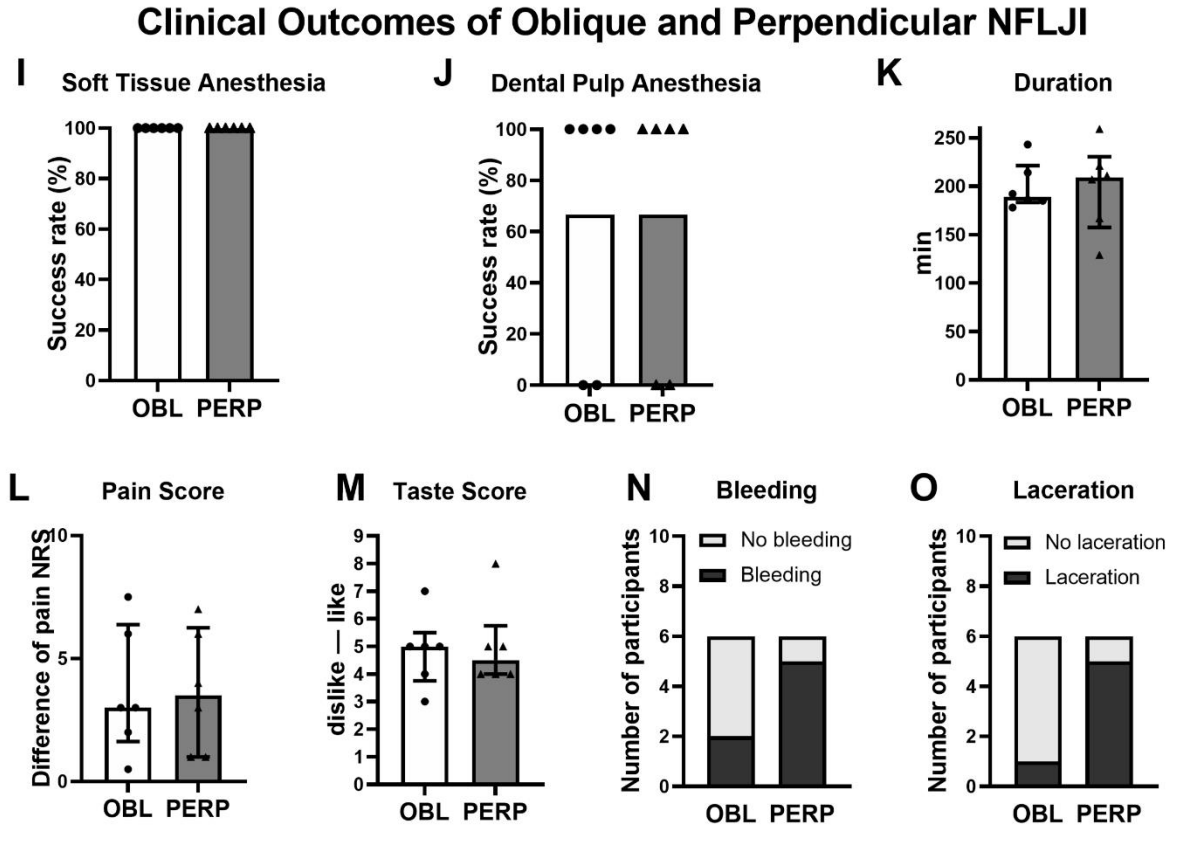
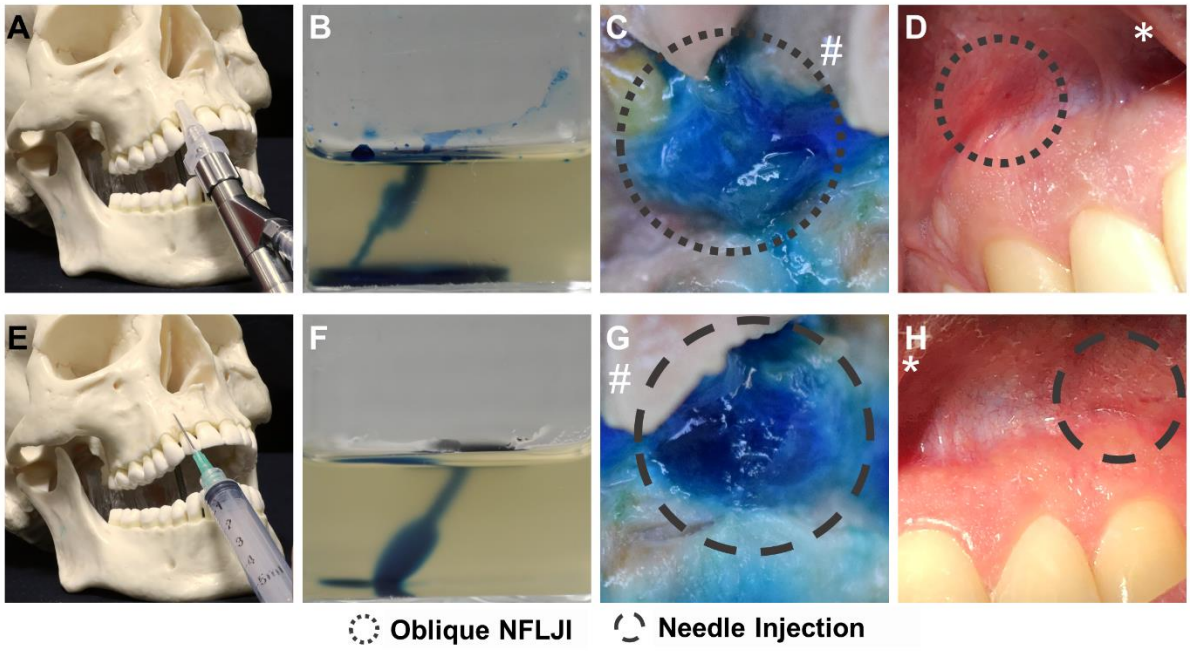

Clinical Outcomes of Oblique and Perpendicular NFLJI
I
Soft Tissue Anesthesia
Success rate (%)
OBL
PERP
J
Dental Pulp Anesthesia
Success rate (%)
OBL
PERP
K
Duration
min
OBL
PERP
L
Pain Score
Difference of pain NRS
OBL
PERP
M
Taste Score
dislike — like
OBL
PERP
N
Bleeding
No bleeding
Bleeding
Number of participants
OBL
PERP
O
Laceration
No laceration
Laceration
Number of participants
OBL
PERP

Figure 5.4 Clinical outcomes of oblique and perpendicular NFLJI. (A) Oblique NFLJI (45°) and (E) perpendicular were performed as shown. Greater fluid regurgitation volume was observed after (F) perpendicular NFLJI than after (B) oblique NFLJI on cadavers, hence more fluid dispersed underneath mucosa in (C) oblique NFLJI than in (G) perpendicular one. (H) Perpendicular NFLJI created mucosa laceration while (D) oblique NFLJI did not create laceration (# marks the nose, * marks the superior labial frenulum). (I-O) Descriptive analysis for oblique NFLJI and perpendicular NFLJI. Data presented as percentage or median (IQR) (n=6).



Clinical Outcomes of Oblique NFLJI and Needle Injection

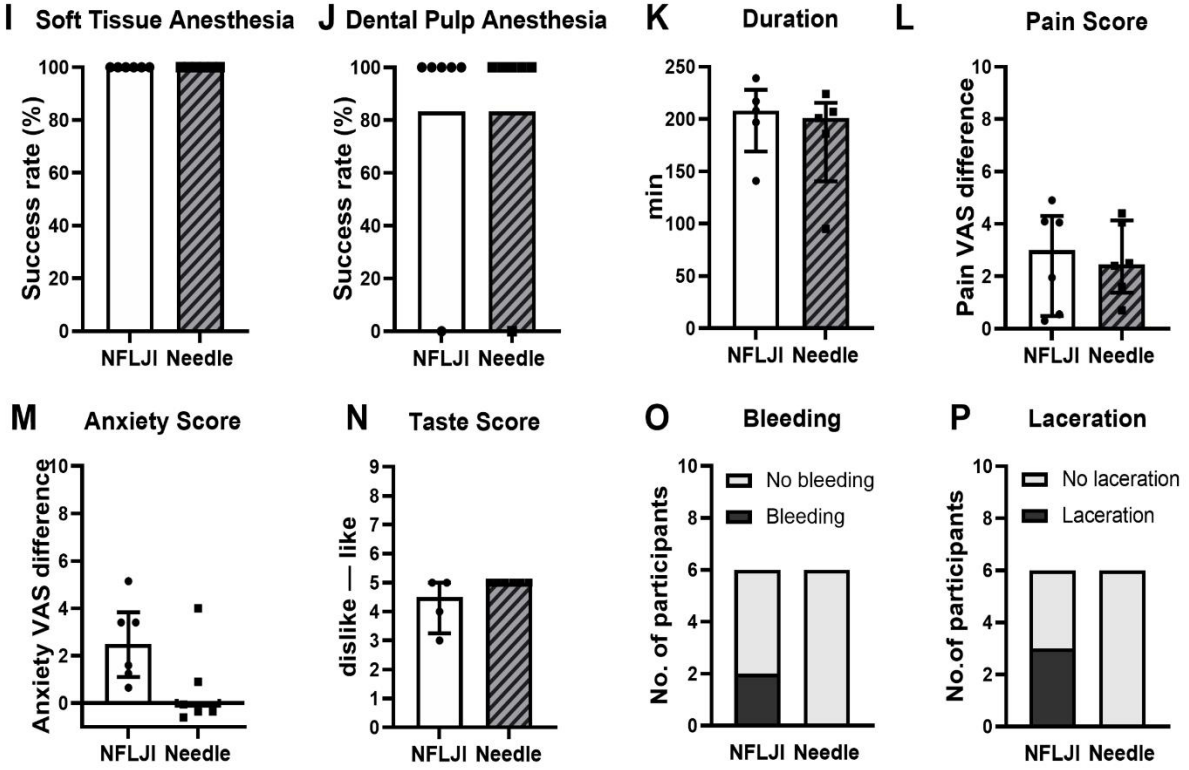


Figure 5.5. Clinical outcomes of oblique NFLJI and needle injection for infiltration anesthesia. (A) Oblique NFLJI and (E) needle injection. Dispersion in soft tissue phantom for (B) oblique NFLJI and (F) needle injection, using 0. 3ml 0.2 % methylene blue. The dispersion in cadavers' upper lateral incisor region, for (C) oblique NFLJI and (G) needle injection. The injection site after (D) oblique NFLJI and after (H) needle injection (# marks the nose, * marks the superior labial frenulum). (I-P) Descriptive analysis for the clinical outcomes of oblique NFLJI group and needle injection group. Data presented as percentage or median (IQR) (n=6).

5.6 Discussion

This study advanced the understanding of how the NFLJI behaves in dentoalveolar tissue and why it caused clinical complications. The perpendicular NFLJI "rebounds" on hard tissue in the dentoalveolar region, resulting in regurgitation and lacerations, which can be mitigated using an oblique injection technique. Moreover, our pilot study demonstrated that it is feasible to conduct a formal randomized trial comparing the clinical efficacy between NFLJIs and needle. The preliminary success rate of infiltration anesthesia is 83.3% for oblique NFLJIs and needle injection.

5.6.1 Oblique and perpendicular NFLJIs

Our study shows that the NFLJI impact angle is crucial, as oblique NFLJIs caused less regurgitation and smaller puncture wound than perpendicular NFLJIs *in vitro* and showed a relatively low risk of clinical complications.

The fluid regurgitation could be explained by the momentum of the liquid stream. In the dentoalveolar region, we observed that NFLJIs penetrated soft tissue easily but bounced back on hard tissues, as explained by the interface defeat phenomenon (*156*). Thus, in perpendicular NFLJIs, the injection stream and the subsequent vertical backflow collide, which reduced the momentum of the incoming stream, therefore causing significant fluid regurgitation and decreasing the volume dispersed underneath the soft tissue (Figure 2G). However, in oblique NFLJIs, the presence of a transverse momentum component along with slightly reduced vertical momentum resulted in horizontal fluid dispersion between the soft tissue and bone surface and caused less fluid regurgitation (Figure 2G). This finding coincides with a previous simulation that studies fluid splash when a high-speed liquid drop impacts a solid surface at different angulations (*157*).

Two theories could explain the puncture wound. On the one hand, perpendicular NFLJIs created larger puncture wounds on tissue phantoms than oblique injections, probably due to the collision between the perpendicular stream and its backflow in perpendicular NFLJIs. On the other hand, the wound size was smaller in oblique NFLJIs, probably resulting from the higher surface pressure of the oblique jet and the lower energy needed to penetrate the soft tissue phantom.

The *in vitro* findings were confirmed clinically, as perpendicular NFLJI caused 5 (83.3%) laceration cases while oblique NFLJI caused 1 (16.7) laceration case. Tissue laceration is a wound produced by tearing due to a blunt force (*158*). In our result, perpendicular NFLJIs caused more lacerations than oblique NFLJIs, presumably due to the greater regurgitation flow and the higher vertical momentum of perpendicular NFLJIs, as shown in figure 2 D& E.

5.6.2 Oblique NFLJI and needle injections

Previous studies comparing NFLJI with needle injections reported inconsistent results (Appendix Table 1), probably due to the inconsistencies in method, such as injection angle, anesthetic dosage and type, and topical anesthesia (*14, 15*). Our pilot randomized clinical trial showed that both oblique NFLJI and needle infiltration anesthesia achieved 83.3% success rates. Local anesthetics work by blocking the sodium channel of nerve cells, thereby blocking the pain signal transfer (*27*). The anesthesia efficacy and duration depend on dosage (volume, concentration) and type (potency, ionization constant, degree of protein binding) (*12*). Theoretically, if both groups deliver anesthetics with the same dosage and type to the same location with the same depth, the anesthesia efficacy and duration should be comparable.

One study reported greater preference for the needle injections than NFLJIs (*17*). This preference may be due to the anxiety when receiving a novel procedure. Indeed, our data showed

that NFLJIs tended to cause a higher anxiety score than the more familiar needle injections, and a few participants verbally expressed their fear of the unknown procedure. In addition, the noise made by NFLJIs might also increase anxiety. A few participants complained about the noise, as corroborated by in previous studies (*16, 17*).

In the first pilot trial, oblique NFLJI induced 1 (16.7%) case of laceration, whereas, in the second pilot trial, it induced 3 (50%) cases. Among the three laceration cases in the second pilot study, two participants moved their heads during the injection. If these two cases were discounted, the actual laceration prevalence for oblique NFLJI would be 16.7% in the second trial. Although oblique NFLJI is considered relatively safe, it still presented a laceration risk of 16.7%. Further research is needed to reduce this risk.

5.6.3 The clinical success rate of infiltration anesthesia on maxillary teeth

Infiltration anesthesia is suitable for managing dental treatment pain on maxillae. The clinical success rate of infiltration anesthesia can be improved by increasing the anesthetics volume (*31*), concentration or potency. For example, 4% articaine has a higher success rate and duration than 2% lidocaine at the same dose since its concentration and potency is higher (*159*). However, evidence showed that anesthetics with higher concentration or potency are associated with a higher risk of nerve paresthesia (*160, 161*).

Our study injected 1mL 2% lidocaine with 1:100,000 epinephrine at the upper lateral incisor region, and the preliminary success rate is 67.7% to 83.3%. These success rates are within the range indicated by previous literature. In previous clinical trials, the clinical success rates ranged from 56.5% to 95% for maxillary infiltration anesthesia using 2% lidocaine with epinephrine, and the weighted average efficacy was 70.3% when the volume is below 2 mL (Table 3) (*162*) (*163*) (*164*) (*165*) (*166*) (*167*) (*168*) (*169*). A study investigated the infiltration

anesthesia at the same injection site as our trial (upper lateral incisor) achieved success rate of 62% on 40 participants using 1.8mL of 2% lidocaine with 1:100,000 epinephrine(*163*). Another study investigating infiltration anesthesia at the canine region using a similar volume as our study (0.9mL 2% lidocaine with 1:100,000 epinephrine), reported a success rate of 64% on 25 participants(*31*).

Table 5.3 The clinical efficacy of infiltration anesthesia on maxilla using 2% lidocaine.

Reference	Anesthetic	Volume	Success	Total	Region	Efficacy
(*162*)	2% lidocaine with 1:80,000 epinephrine	2mL	29	50	Premolars and molars	58%
(*163*)	2% lidocaine with 1:100,000 epinephrine	1.8mL	25	40	Lateral incisor	62%
			29	40	First molar	72%
(*31*)	2% lidocaine with 1:100,000 epinephrine	0.6mL	13	25	Canine	52%
		0.9mL	16	25		64%
		1.2mL	25	25		100%
(*164*)	2% lidocaine with 1:100,000 epinephrine	3.6mL	19	21	Premolars and molars	90.4%
(*165*)	2% lidocaine with 1:100,000 epinephrine	1.8mL	26	32	First premolar	81.25%
(*166*)	2% lidocaine with 1:100,000 epinephrine	3.6mL	24	28	Molars	85.7%
(*167*)	2% lidocaine with 1:100,000 epinephrine	2.1mL	49	55	Premolars and molars	89.1%
(*168*)	2% lidocaine with 1:80,000 epinephrine	1.8mL	13	23	First molar	56.5%
(*169*)	2% lidocaine with 1:100,000 epinephrine	1.8mL	35	40	First molar	88%
		2.3mL	38	40		95%
Total events	2 % lidocaine with epinephrine	≤2mL	211	300	Maxillary lateral incisor to molars	70.3%
		>2mL	130	144		90.3%
		All	341	444		76.8%

5.6.4 Strengths, limitations, and future directions

Ballistic gelatin was chosen to test NFLJIs mainly because its density resembles that of human soft tissues (*170, 171*). However, given that the fracture toughness and porosity of gelatin is different from that of human soft tissue, a novel oral soft tissue phantom material with comparable properties would be desirable. Nonetheless, our cadavers and clinical studies confirmed the findings of our *in vitro* experiments, which supported the use of ballistic gelatin as a model for NFLJI research.

Our study reported a systematic methodology for developing and translating NFLJI techniques from the laboratory to clinical practice, and out pilot RCTs confirmed the safety and feasibility for conducting a larger non-inferiority RCT in the future. However, the limited sample size in two pilot RCTs does not allow assessment of differences between injection techniques. Therefore, the differences we observed clinically between perpendicular and oblique NFLJI should be interpreted with caution. A future RCT with larger sample size would be needed to assess the efficacy and safety of the optimized NFLJI infiltration techniques with enough statistical power.

Previous randomized controlled trials evaluating dental anesthesia efficacy used a sample size of 40-50 patients to detect a difference of 25% (α= 0.05, β= 0.2) (Kanaa et al. 2012) (Evans et al. 2008). However, a non-inferiority randomized controlled trial with a parallel-group design (*54*) and with a 5% non-inferiority margin (*172*), would require a total of 2300 participants to test the hypothesis (α = 0.05, β= 0.2, success rate 70.3%, withdrawal rate= 10%) (*173*). This sample size is not easy to achieve.

A non-inferiority randomized controlled trial using a cross-over design could have sufficient power with 160 to 492 participant based on statistical simulation (α= 0.05, β= 0.2)

(*174*). Data from such study design must be analyzed with a mixed model logistic regression. Future studies using this design should report odds ratios of efficacy and frequencies of concordant-discordant results per group to allow further calculations for power or sample size as well as meta-analyses.

5.7 Conclusion

Oblique NFLJI could be a promising approach for dental infiltration anesthesia, causing minimum drug regurgitation with a relatively low risk of complication. The pilot randomized controlled trial showed that it would be feasible to conduct a non-inferiority randomized control trial with a cross-over design to test whether the efficacy and effect of oblique NFLJIs are comparable to those of needle injections.

Acknowledgments: We sincerely thank Robert L'Heureux B.S., Jamie Brisebois B.S. from the Department of Cell and Anatomy, McGill University, for organizing the cadaver experiments. We also appreciate the generosity of the body donors and their families. Our gratitude further extends to Dr. Nathalie Morin, Rosa Menale, and Ann Marie Plante, from the Faculty of Dentistry, for supporting our clinical trials; to Dr. Xiangda Cui for advising the fluid dynamic mechanism, and to Dr. Yin Zhen for drawing the figure in the graphic abstract.

Funding: This research was sponsored by the Natural Sciences and Engineering Research Council of Canada (543972-19 and 366077487). The first author was sponsored by the Clifford C.F. Wong Fellowship, réseau de Recherche en Santé Buccodentaire et Osseuse and Alpha-Omega Foundation of Canada from McGill University, and the Doctoral fellowship from the Chinese Scholarship Council.

Author contributions: Q. GAO contributed to the conception, study design, data acquisition, and interpretation of the study, drafted and revised the manuscript. D. TAQI, M. ABUSAMAK,

K. MENASSA, and A. HENLEY contributed to data acquisition, and revision of the manuscript. G. NOEL, Z. D. KHATCHADOURIAN, A. VELLY, E. ELHAM, L. MONGEAU, and F. TAMIMI contributed variously to the conception, design, data analysis, interpretation, and revision of the study, F. TAMIMI critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of the work. The authors declare that there is no conflict of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Supplementary Table 5.1. Literature review of all NFLJI dental anesthesia clinical studies. Search terms: "needle-free injection or jet injection" and "dental anesthesia" and "clinical trial," between Jan1 1979 and April 1st, 2020, article in English; Conclusion: These five papers compared the outcomes between needle-free and needle dental infiltration anesthesia. The efficacy of needle-free anesthesia is remaining unclear and poorly investigated. The technique for needle-free devices is poorly described and discussed in those papers(14-17, 62).

Ref.	Design (procedure, sites)	Patients (sample size)	NFL JI	Agent	Method of injection	Main Outcomes (NFLJI vs. needle)
(Dabarakis et al., 2007)	Two arm design (infiltration, NR, NR)	Adult (n=22)	INJEX	0.3mL 2% lidocaine with epinephrine	Perpendicular injection	• Efficacy is equal (data not reported) • Time of onset is similar (data not available) • Duration of anesthesia is shorter (data not available) *
(Arapostathis et al., 2010)	Split-mouth design (infiltration, anterior teeth, and upper molars)	Children (age 6-11) (n=87)	INJEX	0.4mL articaine 4% with epinephrine (used topical gel)	NR	• Acceptance or Preference are reduced (12.6% vs. 73.6%) * • Effectiveness is reduced (additional anesthesia 80.5% vs. 2.3%) * • Pain during anesthesia is greater (70.1% vs. 4.6%) * • Fear during anesthesia is increased (81.6% vs. 13.8%) * • Complications are increased: bleeding (60.9% vs. 26.4%) *; bad taste (56.3% vs. 9.2%) *; stinging (46.0% vs. 6.9%) *; discomfort (18.4% vs.9.2%) *
(Makade et al., 2014)	Split-mouth (infiltration, premolar and molar region)	Adult (n=20)	MadaJet	0.4 mL 2% lidocaine with 1:80,000 epinephrine (used topical gel)	NR	• Acceptance or Preference is greater (70% Vs 20%) * • Efficacy is equal (EPT results were not reported) • Pain is reduced (1.65± 0.93 vs 3.55± 1.67) * • Fear is reduced (5.15± 3.18 vs. 1.6± 1.6) * • Discomfort during injection is greater (2.55± 1.35 vs. 0.55± 0.76) * • Time of onset is faster (21± 6.20 vs.48.2± 20.85s) * • Duration is shorter (20.75 ± 3.53 vs 50 ± 9.32 min) * • Bad taste tends to be greater (1.8± 1.36 vs.1± 1.45)
(Oliveira et al., 2019)	RCT, split-mouth (infiltration, maxillary first molar)	Adults (n=41)	Comfort-in	1mL Lidocaine 2% with epinephrine 1:100,000	Perpendicular injection	• Pain score during injection is similar [12.2(0-55.4) Vs 12.1(0-53.8)] • Latency time for anesthesia is similar (2 vs. 2 min) • Duration of pulp anesthesia is shorter (Mean: 20 vs. 40min) * • Efficacy is equal: no patients required additional anesthesia
(Ocak et al., 2019)	Split-mouth (infiltration, premolars)	Adolescent for orthodontic treatment	INJEX	0.4mL	Perpendicular injection (3000psi)	• Pain score during injection is reduced (1.5± 1.8 Vs 3.14±2.01) * • Pain score during procedure is grater (3.86± 3.23 Vs 2 ±2.05) * • Efficacy is reduced (additional injection 28.6% Vs7.1%) *

	(n=28)	4% articaine with 1:200,000 epinephrine	• Bad taste is greater (3.36 ±2.38 Vs 2.11± 2.18) * • Duration is shorter (40.89±17.32 Vs 64.46±27.93 min) *

INJEX: INJEX Pharma, Berlin, German; MadaJet: MADA Medical Products Inc, New York, USA; Comfort-in: Mika Medical Inc, Busan, Korea

* means $p<0.05$, there was a significant difference of this variable among needle-free and needle group; NR, data was not reported in full text.

Supplementary Table 5.2 Continuing to Appendix table 1, the clinical efficacy of needle-free infiltration anesthesia compared to needle infiltration anesthesia.

Reference	Anesthetic	Method	Volume	Success	Total	Region	Efficacy
(Dabarakis et al., 2007)	mepivacaine 3%	NFLJI	0.3mL	0	10		0%
	lidocaine 2% with epinephrine 1:80,000	NFLJI		14	22		63.6%
		Needle		NA	14		NRE
(Arapostathis et al., 2010)	4% Articaine with 1:200,000 epinephrine	NFLJI	0.4mL	17	87	Upper molars anterior teeth	19.5%
		Needle		85	87		97.7%
(Makade et al., 2014)	2% lidocaine with 1:80,000 epinephrine	NFLJI	0.4mL	NA	20	Premolar and Molar region	NRH
		Needle		NA	20		
(Oliveira et al., 2019)	Lidocaine 2% with epinephrine 1:100,000	NFLJI	1mL	41	41	Maxillary molars	100%
		Needle		41	41		100%
(Ocak et al., 2019)	4% Articaine with 1:200,000 epinephrine	NFLJI	0.4mL	20	28	Premolars	71.4%
		Needle		26	28		92.8%
Total events		NFLJI	<0.5mL	51	137		37.2%
		Needle		111	115		96.5%
		NFLJI	1mL	41	41		100%
		Needle		41	41		100%
		NFLJI	All	92	178		51.6%
		Needle		152	156		97.4%

NR, the data was not reported in the full text.

NRE, data was not reported in the full text, but efficacy was reported to be equal between two group.

NRH, data was not reported in the full text, but efficacy in the needle group was higher than needle-free group.

Chapter 6 Needle-free injection: mental incisive nerve block

6.1 Preface

This chapter investigated the high-speed liquid jet for mental incisive nerve block (MINB), which requires thicker penetration depth to deliver anesthetics to the major nerve branches. The relationship between penetration depth and NFLJI parameters was investigated. A systematic *in vitro* experiment was designed to evaluate the safety of NFLJIs based on their force-time and depth-time history using different supply pressure. The maximum injection force, total work, jet impingement pressure, and jet penetration pressure were compared between high- and low-pressure NFLJIs and compared to needle injections. NFLJI technique for MINB evaluated on cadavers, then validated in two pilot randomized controlled trials for its safety and feasibility.

This chapter(*175*) has been published at the "international journal of pharmaceutics".

Gao, Q., Henley, A., Noël, G., Der Khatchadourian, Z., Taqi, D., Abusamak, M., He, Z., Groen, S., Taher, R., Menassa, K. and Velly, A., 2021. Needle-free Mental Incisive Nerve Block: In vitro, Cadaveric, and Pilot Clinical Studies. International Journal of Pharmaceutics, 609, p.121197.

https://doi.org/10.1016/j.ijpharm.2021.121197

Needle-free Injection: Mental Incisive Nerve Block *in vitro*, cadaveric, and pilot clinical studies

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Abstract word count: 198, total word count: 6871.
Number of figures: 6. Number of tables: 4.
Number of supplementary figures:5. Number of supplementary tables: 2
Number of References: 49.

This study has been registered online with the title needle-free dental anesthesia (NCT04493528), https://clinicaltrials.gov/ct2/show/NCT04493528.

Highlight of the paper:

- Needle-free liquid jet penetrates deeper with increased pressure or volume.
- Low-pressure needle-free liquid jet injections are safer than high-pressure ones.
- It is feasible to conduct a non-inferiority cross-over randomized controlled trial.

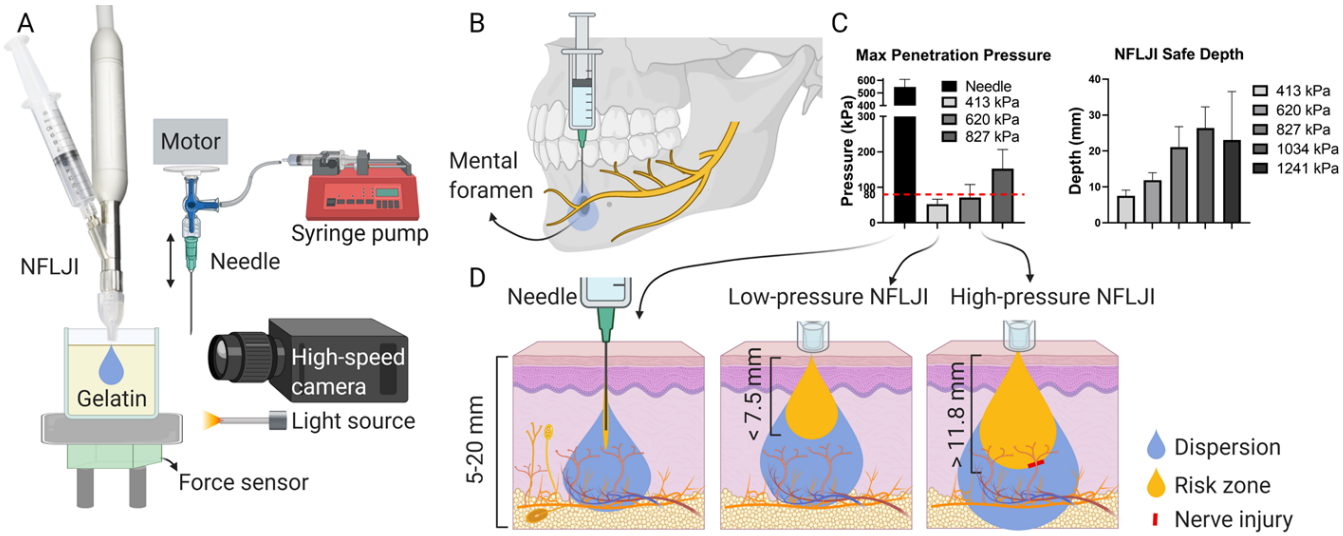


Figure 6.1 Graphic Abstract

6.2 Abstract

The present study aimed to optimize Needle-Free Liquid Jet Injection (NFLJI) for Mental Incisive Nerve Blocks (MINB) and evaluate its clinical safety and feasibility. A MINB protocol was developed and optimized by series of NFLJI experiments in soft tissue phantoms and cadavers, then validated in two pilot Randomized Controlled Trials (RCT). The NFLJI penetration depth was found to be directly proportional to the supply pressure and volume. High-pressure NFLJIs (620 kPa or above) created maximum force and total work significantly greater than needle injections. Low-pressure NFLJIs (413 kPa), however, produced results similar to those of needle injections. Additionally, high-pressure NFLJIs created jet impingement pressure and maximum jet penetration pressure higher than the results of low-pressure NFLJIs. Pilot RCTs revealed that high-pressure NFLJI caused a high risk of discomfort (60%) and paresthesia (20%); meanwhile, low-pressure NFLJI was less likely to cause complications (0%). The preliminary success rates of MINB from cadavers using NFLJIs and needles were 83.3% and 87.5%. In comparison, those from RCTs are 60% and 70%, respectively. To conclude, NFLJI supply pressure can be adjusted to achieve effective MINB with minimal complications. Furthermore, the cadaver study and pilot RCTs confirmed the feasibility for further non-inferiority RCT.

Key words: Dental Anesthesia; Mental Nerve; Jet Injections; Pilot Studies; Feasibility Studies; Randomized Controlled Trial; Paresthesia.

Abbreviations: NFLJI, Needle-Free Liquid Jet Injection; RCT, Randomized Controlled Trial.

6.3 Introduction

Needle fear and phobia may deter patients from receiving necessary treatment, worsening their oral health conditions (*2-5*). Needle-Free Liquid Jet Injection (NFLJI) systems could solve this problem. These systems are powered by gas (*140*), laser (*176*), or spring (*177*) pressure to create thin (usually 76-360 μm in diameter) and high-velocity (typically >100m s^{-1}) liquid jets. The liquid jets can deliver therapeutic fluid across the skin into the subcutaneous or intramuscular region (*1*). In addition, the use of NFLJI eliminates the risk of needle fracture during injection (*6*) and disease transmission via re-used needles (*1*).

Dental anesthesia is mainly achieved by two different techniques: infiltration and nerve blocks. Infiltration anesthesia is achieved by penetrating through a thin layer of mucosa (3-5mm thick) overlying the rigid alveolar bone and depositing anesthetics near the small nerve terminals of the tooth apices and the surrounding soft tissue (*12*). These anatomical characteristics pose a challenge to NFLJI. Although a high-speed jet can easily penetrate the mucosa, it can rebound off the hard tissues resulting in significant liquid regurgitation and tissue laceration. Recently, our group demonstrated that these problems could be mitigated using the oblique impact angle, which helps achieve adequate infiltration anesthesia with minimal complications (*140*).

Although infiltration anesthesia can adequately anesthetize the maxillary and mandibular anterior teeth, it cannot anesthetize the mandibular posterior teeth because their small nerve endings are embedded deep in partially impermeable bone (*12*). For the latter, nerve block anesthesia is needed. Dental nerve blocks deliver anesthetics to desensitize major nerve branches that control downstream teeth and soft tissues; Unlike infiltration anesthesia, nerve blocks require deeper injections able to penetrate deep enough (5 to 20 mm) to reach the major nerves (*12*). The nerve block technique poses different challenges compared to infiltration anesthesia

due to the anatomical structure. Moreover, the risk of high-speed liquid jets directly impacting main nerve branches remains unclear. However, to the best of our knowledge, NFLJI has not been investigated in depth for nerve block applications.

The mental incisive nerve block (MINB) is a technique used to anesthetize mandibular premolars by injection anesthetic solution near the mental foramen to block the mental incisive nerve (*38, 178, 179*). MINB requires a relatively simple penetration depth of 5-6 mm (*180*) and thus was selected for this study.

Three factors influence NFLJI penetration and dispersion: the injector and operative parameters (*1, 63, 181*), the tissue properties (*64*), and the injected fluid (*65, 182, 183*). Among these factors, only the injector parameters may be adjusted to optimize the outcome. Poor selection of injector parameters can cause undesirable side effects, such as tissue damage and nerve paresthesia. Therefore, appropriate parameters are the most critical consideration for safe NFLJIs before translating the NFLJI to clinical practice.

This study aimed to investigate the NFLJI technique for MINB and to evaluate its clinical safety and feasibility. We hypothesized that the NFLJI penetration depth and potential tissue damage are correlated with the supply pressure; and that an optimal supply pressure could achieve successful MINB with minimal complications.

6.4 Material and Methods

A pneumatic NFLJI system (Meso-Jet/Med-Jet, Medical International Technologies Inc, Montreal, Canada) was used in this study. The system was operated to deliver 0.1-1.8 mL of volume. The air supply pressure was 413-1400 kPa (60-200 psi), and the orifice diameter was 120 μm for all experiments (Figure 6.2 A-C). Note that the supply pressure determines the

acceleration of the free piston inside the system and does not necessarily correspond to the pressure immediately upstream of the nozzle.

6.4.1 Characterization of phantoms for in vitro NFLJI experiments

To develop an appropriate phantom for *in vitro* NFLJI experiments, first, the Young's modulus and fracture toughness of oral soft tissue was quantified using tissue samples harvested from fresh porcine heads within 24 hours post-mortem. Young's modulus is the elasticity of a material measured by a rheometer assessing how it withstands the compression or elongation with respect to its length. Fracture toughness is the ability of a material to resist fracture. Both serve as a basis for material comparison, selection, and quality assurance (*67*). The fracture toughness of many materials is determined by a shear test or through a single-edged notch test with coupon-type specimens. These methods are not applicable for oral soft tissue due to size limitations. Oral soft tissue toughness can alternatively be determined using scissor-cutting tests (*124*) or needle-insertion tests (*125*). The latter method was selected in this study as the test procedure is similar to the needle injection.

To measure Young's modulus, cylindrical porcine oral mucosa samples with a 10-mm diameter and 2-mm thickness were prepared and preserved in a PBS bath (Fig.6.1 C&D). Gelatin phantom samples (Sigma-Aldrich, Merck KGaA, US) with similar dimensions were prepared with a concentration ranging from 2 wt.% to 10 wt.% (*171*) using a mold. The Young's modulus was inferred from the shear modulus, with the assumptions that the tested material is isotropic, homogeneous, and incompressible. Shear tests were performed using a torsional rheometer (DHR2, TA instrument, USA), with a test head diameter of 10 mm, at frequencies from 1-100 Hz (Fig. 6.1 C).

To measure the fracture toughness, oral soft tissue was harvested from three fresh porcine heads. Rectangular samples with dimension 2×4 ×1 cm were prepared using dissection tools and mounted within 5% gelatin inside a 4×4×4-cm glass container (Fig. 6.1F). A 25-gauge needle driven by a motorized linear transverse stepper (SPN7338, Velmex Inc, US) at a velocity of 5 mm/s was inserted into the sample to a 15-mm depth. The needle was retracted and inserted a second time at the same location to evaluate friction forces. A force transducer (GS0500, transducer technique, USA) located underneath the glass container recorded the vertical force-time history during needle insertion (Fig. 6.1F). LabVIEW (LabView 2019, National Instruments, US) was used to program the needle movement and record the force data. The fracture toughness of porcine masseter muscles and abdominal muscles, as well as the gelatin of 5 wt.% and 10 wt. % were also quantified using the same method for oral mucosa. The fracture toughness was calculated using the relation (*127*):

$$\int_{x2}^{x1}(F - F')\mathrm{d}x = J_{IC}\ a\ \mathrm{d}x \qquad Eq\ (1)$$

where $x1$ and $x2$ are the beginning and end positions of the needle insertion, F is the dynamic force during the first insertion (friction + fracture), F' is the dynamic force during the second co-located insertion (friction alone), a is the cross-sessional area of the needle, and $\mathrm{d}x$ is the dynamic change of needle position. The fracture toughness, J_{IC}, could be calculated from Eq (1) (*184*).

6.4.2 Laboratory investigation of NFLJI safety

According to the test results and a previous study (*185*), 5 wt.% gelatin can best represent Young's modulus of oral soft tissue. Hence 5 wt.% gelatin was prepared in customized optical clear glass containers of W 4×L 4× H (4-15) cm dimension for further NFLJI test. To investigate how NFLJI parameters affect injection in oral soft tissue, NFLJI experiments were conducted in

gelatin phantom using a range of supply pressure (413-1240 kPa) and delivery volume (0.1-1 mL). The jet travel in the air was also recorded using a high-speed camera (Fastcam MC2, Photron, Japan) (*170*) to estimate the initial liquid jet velocity. The NFLJI nozzle tip was 2 mm from the phantom surface to maintain a visible jet trajectory for high-speed camera analysis. The NFLJI impact angle is 90° to the phantom surface to maintain sufficient phantom thickness.

A laboratory test bench was designed to simultaneously measure the force-time history during injection using a force sensor and the jet dispersion-time history using a high-speed camera (Fig. 6.1A) to investigate the relationship between injector parameters and tissue damage. Afterward, this setup was modified to simulate clinical needle injection by adding a linear stage (SPN7338, Velmex Inc, US) and a syringe pump (NE-1000, New Era pump system Inc) (Fig. 6.1B) to measure the dynamic force during needle injection.

High-speed videos were recorded at 10,000 frames per second (fps) and analyzed frame by frame to plot the penetration depth-time history and match with synchronously acquired force-time history. Force data were processed using MATLAB (The MathWorks, Inc, USA). Figures were refined using Prism8 (GraphPad Software, USA). The maximum force (Fig. 6.3A) was determined as the highest force value during the NFLJI or needle injections, as shown in Fig. 6.3 A, B.

The total work of NFLJI was calculated as the integral of dynamic force (F) and dynamic penetration depth of the jet leading edge (x) versus time from the beginning ($x1$) to the end position point ($x2$), i.e.

$$W = \int_{x2}^{x1} F \, \mathrm{d}x \qquad \text{Eq (2)}$$

The total work of needle injection was the sum of the calculated work for needle insertion ($W_{insetion}$) and the estimated work for the injection of 1-mL fluid ($W_{injection}$). The needle insertion work was calculated as the integral of dynamic force (dF) multiplied by needle travel (dx) at each sampling interval. The estimated work of injection was calculated as the product of injection volume (V), the minor loss coefficient for the flow through the needle (K, $K = 1$ in this case), the density of water at 20 °C (ρ), and average velocity of fluid flow ($\bar{U}$), which is based on Euler's equation for the kinetic energy of fluid (*186*). The average velocity of fluid flow ($\bar{U}$) was calculated as volumetric flow rate (Q) divided by the internal area of the needle (A_{needle}). The mathematical expressions used are:

$$W = W_{insetion} + W_{injection} \quad , \qquad \text{Eq (3)}$$

$$W_{insertion} = \int_{x_2}^{x_1} F \cdot dx \quad , \qquad \text{Eq (4)}$$

$$W_{injection} = V \cdot K \cdot \frac{1}{2} \rho \bar{U}^2 \quad , \qquad \text{Eq (5)}$$

and $$\bar{U} = \frac{Q}{A_{needle}} \quad . \qquad \text{Eq (6)}$$

The impulses of NFLJI (Fig. 6.3A) and needle injection (Fig. 3B) were calculated as the integral of dynamic force (F) versus time (dt) as

$$I = \int_{t_2}^{t_1} F \cdot dt \quad . \qquad \text{Eq (7)}$$

The duration of NFLJI was the difference between the beginning and ending points of injection in the high-speed video. The duration of needle injection was the difference between the beginning and ending points of the needle movement.

The jet central core velocity was calculated as:

$$v = \frac{\bar{F} \cdot \Delta t}{\rho \cdot V} \quad , \qquad \text{Eq (8)}$$

where $\bar{F}$ is the mean force during jet penetration, Δt is the duration of jet injection, ρ is the density of water, V is the volume of injected liquid.

Further analysis was done after matching the force-time history (Fig. 6.3A) and dispersion-time history (Fig. 6.4E). First, the jet impingement force was defined as the force when jet starts to impinge the phantom surface. The estimated jet impingement pressure was calculated as jet impingement force divided by the skin hole area; the latter was calculated using a nozzle/ skin wound diameter ratio of 0.3 based on a previous study (*64*). Since the jet penetration pressure is highest at the surface and decreases with depth, the estimated jet maximum penetration pressure was calculated as the force when the jet pieces through the tissue phantom (Fig.6.3Aa) divided by the jet dispersion area calculated from the first frame where jet penetration is visible in the high-speed camera video (Fig. 6.4 Ea) (Table S6.1). In the contrast, the maximum force of needle injection occurs at the end of insertion (Fig. 6.3B); hence the maximum pressure of needle injection is calculated by the maximum force when the needle penetrates the tissue phantom divided by the area of a 25-gauge needle.

A previous study has determined the safe pressure for nerve damage is 80 kPa (*187*). Accordingly, the safe depth of NFLJI was defined as the depth beyond which the jet pressure inside the tissue phantom drops below 80 kPa.

6.4.3 Cadaveric evaluation for the efficacy of NFLJI mental nerve block

A total of ten cadavers were used. Two cadavers were used to validate the NFLJI parameters for MINB. Eight cadavers were used for a randomized cross-over split-mouth study to compare the anesthesia efficacy between NFLJI and needle. Methylene blue (0.2%) was used to visualize the injection outcome (*130*) using a volume of 0.3 mL (*65*).

Needle injection MINBs were performed following standard procedures (*12*) (Fig.6.2G). Needle-free MINBs were performed by placing the nozzle of the NFLJI device in the mucobuccal fold of the mandibular vestibule using a mean loading force of 0.3 N at the premolar region and depositing the local anesthetic around the mental foramen (Fig. 6.2 J). After each injection, the site was dissected by an independent anatomist (G.N.) and photographed.

In cadaveric studies, the typical evaluation for the success of nerve blocks is based on staining patterns. Unfortunately, this evaluation is subjective and inaccurate. To address this issue, we added additional objective criteria: the mental nerve was adequately stained (*131*), the mental foramen was in the center of the stained area (*132*), and four blinded assessors agreed on the judgment (Fig. 6.2 H&I, K&L). In addition, the four blinded assessors had to be experienced dentists or anatomists.

6.4.4 Clinical validation of high-pressure NFLJI

A pilot RCT with a split-mouth cross-over design was conducted at the McGill Student and Staff dental clinic over September 1-20, 2019, to evaluate the feasibility and safety of high-pressure NFLJI for MINB. This study was approved by the McGill Research Ethics Board (A09-M36-18A) and retrospectively registered online (NCT04493528). Ethical approval for the clinical and cadaver studies was obtained under the same ethical protocol because experiments were considered two stages of the same study. The clinical investigation performed in this study followed a similar methodology to our previous work regarding the inclusion and exclusion criteria, endpoint, allocation, randomization, blinding, and follow-up (*140*). However, the six participants enrolled are different from the previous study.

Each participant received two MINBs on the left and right lower premolar regions, one site with needle injection and another with NFLJI. The injection techniques were the same as

described in the cadaveric study. The NFLJI supply pressure was 620 kPa. As justified previously, the anesthetic was 1 mL of 2 % Lidocaine with 1:100,000 epinephrine (*140*).

The primary outcomes were feasibility and safety: the feasibility depended on recruitment time and rate, withdrawal rate, participants' concerns, and problems during operation, while the safety of NFLJI was determined by complications such as bleeding, laceration, hematoma, and nerve paresthesia. The secondary outcomes were recorded in three categories: (a) the success rate of MINB, confirmed by electric pulp test (EPT) on canine, premolars, and first molar (*12*); (b) the effect of MINB, including the time to initial anesthesia, the onset, and the duration; (c) side effects after injections, including pain score assessed using the numeric rating scale (*149*) and taste score measured using the 9-point hedonic scale (*150*) (Fig. S6.1 A).

6.4.5 Clinical safety and feasibility of low-pressure NFLJI

The first pilot RCT was stopped due to one case of paresthesia; the *in vitro* experiment suggested that reducing the supply pressure could reduce complications by minimizing the total work, force and pressure applied on the soft tissue. For these reasons, a second pilot clinical trial was conducted to validate the safety of the refined NFLJI technique using a lower supply pressure of 413 kPa. Another 6 participants were recruited, and the clinical trial was conducted from January 6 to March 12, 2020, at the McGill Student and Staff Dental Clinic. The study design, primary outcomes, and secondary outcomes were the same as the first pilot RCT (Fig. S6.1 B). In addition, a visual analog scale (VAS) for anxiety was added to assess the anxiety levels before and during the injections; the pain numeric rating scale (NRS) was replaced by a pain VAS to provide a more sensitive measurement (*153*).

6.4.6 Statistical Analysis

Descriptive analysis was performed using SPSS21.0 (IBM, SPSS statistics) and Prism8 (GraphPad Software, San Diego, California, USA). Categorical variables, such as complication rates and success rates, were presented as count and percentage. Continuous variables, such as durations and scores, were presented as median and inter-quartile range (IQR).

6.5 Results

6.5.1 NFLJI penetration depth and its parameters

Gelatin of 5 wt. % was selected for *in vitro* experiments as its Young's modulus is similar to that of porcine oral soft tissue (Fig. 6.1E), even though gelatin's fracture toughness is significantly lower than that of oral soft tissue (Fig. 6.1G).

The time history of the liquid jet dispersion in air and in gelatin are shown in Fig. 6.2 D&E. Upon impingement on the soft tissue phantom surface, the jet penetrates the surface and then creates an initial conical region of high velocity. Over time, flow recirculation accumulates at the end of the conical tunnel to create a pocket-like region with increased width. This process would be repeated within the initial pocket-like region resulting in a secondary conical tunnel and pocket-like propagation (Fig. 6.2E). Higher shear between the injected liquid and the solid substrate causes fractures and breakages of the substrate into a slurry. Vorticity accumulation at the end of the conical tunnel results in large-scale flow recirculation. The recirculated flow region acts as a drill, carving deeper into the substrate over time. Eventually, momentum decreases, and shear is reduced so that the injected drug simply diffuses into the solid substrate with no visible fracture.

In vitro assessment of NFLJI revealed that the penetration depth (D) is directly proportional to the delivery volume (V) and supply pressure (P) (Fig. 6.2 F) according to the relation:

$$D = 10.58 + 17.93V + 0.03P \quad , \qquad \text{Eq (9)}$$

(This equation is only valid for the following threshold 413 kPa < P <1241 kPa, 0.1 mL< V < 1 mL).

where V is the volume (mL), P is the pressure (kPa), D is the depth (mm), and the coefficient of determination for this linear model is 75 % ($r^2 = 0.75$).

6.5.2 In vitro analysis of NFLJIs

The maximum force of NFLJI and total work were measured and calculated, they were found to be directly proportional to the supply pressure (Fig. 6.3 C D). For NFLJIs employing supply pressure from 413 kPa to 1241 kPa and volume of 1 mL, the mean (SD) maximum force was 0.11 (0.034) N to 0.37 (0.036) N (Fig 6.3C), total work was 0.0024 (0.00048) J to 0.015 (0.0017) J (Fig. 3D). For NFLJIs employing 1 mL and pressure from 413 to 1241 kPa, the mean (SD) duration was found to be 0.70 (0.16) s to 0.50 (0.02) s, and this duration showed a reducing trend when the pressure increases (Fig. 6.3F). Based on the force-time history and Eq (7), the mean (SD) impulses were from 0.072 (0.010) $N \cdot s$ to 0.11 (0.014) $N \cdot s$ (Fig. 6.3 E).

Further analysis was done by matching the force-time history from the sensor and depth-time history from the high-speed camera. For supply pressures from 413 kPa to 1241 kPa and volume of 1 mL, the NFLJI mean (SD) central stream velocity increases from 55.9 (28.4) m/s to 162.5 (15.3) m/s (Fig. 6.4A), the estimated jet impingement pressure increased from 706.4 (250.8) kPa to 2530.0 (296.5) kPa (Fig. 6.4B). The estimated maximum jet penetration pressure was from 52.5 (14.1) kPa to 148.1 (73.4) kPa (Fig. 6.4C). These three variables were found to be directly related to the supply pressure. The mean (SD) estimated safe depths were 7.5 (1.6) mm to 23.1(13.5) mm (Fig. 6.4D). An example of pressure estimation was shown in Table S 6.1.

6.5.3 In vitro analysis of needle injections

For delivery flow rates of 1.8, 3.6, 7.2 mL/min and volume of 1 mL, the needle injections created a mean (SD) maximum force of 0.078 (0.0085) N, 0.077 (0.0083) N, and 0.071(0.0068) N, respectively (Fig. 6.3C). Since the insertion speed of needle is 5mm/s, there is no significant difference among the maximum force of needle injections. The total work of needle injection were 0.0014 (0.00011) J, 0.0019 (0.00007) J, and 0.0036 (0.00008) J, respectively for the three

different flow rates (Fig. 6.3 D). As for the duration, needle injections of 1 mL fluid using flow rates of 1.8, 3.6, 7.2 mL/min, showed respective mean (SD) durations of 40.5 (0.17) s, 24.3 (0.26) s, and 18.1 (0.26) s. Based on the force and time history, the respective impulse for needle injection with the above-mentioned flow rates were 2.1(0.24) $N \cdot s$, 1.2(0.16) $N \cdot s$, and 0.77 (0.07) $N \cdot s$. The maximum force of needle injection occurs at the end of insertion (Fig. 6.3B), leading to a mean (SD) estimated maximum penetration pressure of 527.2 (56.1) kPa.

6.5.4 In vitro comparison between NFLJIs using high or low pressure, and needle injections

High-pressure NFLJI (620 kPa or above) resulted in maximum force and total work values that were significantly higher than the values of needle injections. Low-pressure NFLJIs (413 kPa), however, featured total work and maximum force similar to those of needle injections (Fig. 6.3 C&D). Needle injections conversely induced impulse and duration significantly higher than those of NFLJIs (Fig. 6.3E&F) since the impulse value is directly proportional to the duration.

Upon impinging the soft tissue phantom, low-pressure NFLJI (413 kPa) created a mean (SD) jet impingement force of 0.089 N (0.031) resulting in a mean NFLJI impingement pressure of 706.4 (224) kPa, while the high-pressure NFLJI (620 kPa) created a mean jet impingement force of 0.14 (0.027) N and therefore a mean jet impingement pressure of 1149.8 (194.7) kPa (Fig. 6.4B). Besides, once the jet penetrated through the phantom surface and started to travel inside, the low-pressure NFLJI resulted in a maximum penetration pressure of 52.46 (14.09) kPa, which is always below 80 kPa; While the high-pressure NFLJI created a maximum penetration pressure of 71.25 (36.66) kPa, indicating a higher risk of nerve damage (Fig. 6.4 BC). Needle injections created a maximum penetration pressure of 527.2 (56.1) kPa, which was lower than

the jet impingement pressure [706.4 (250.8) to 2530.0 (296.5) kPa], but higher than the maximum jet penetration pressure [52.5 (14.1) to 148.1 (73.4) kPa].

Low-pressure NFLJI had a mean safe depth less than 7.5 (SD 1.4) mm, while high-pressure NFLJI had a mean safe depth above 11.9 (SD 1.9) mm (Fig. 6.4D). Since there is always a risk of needle tip piercing the nerve for needle injections, there is no safe depth for needle injections.

6.5.5 MINB using NFLJI on cadavers

A total of twenty MINBs were performed on ten cadavers. Twelve injections were performed using NFLJIs (0.3mL, 120 psi), and eight injections were performed using needles. The simulated success rates of MINB were 83.3% in the needle group and 87.5% in the NFLJI group. No significant difference was found between the two methods regarding the efficacy of MINB (Table 6.1) (Fig. 6.2 M&N)

Table 6.1 The success rate of MINB using needle or NFLJI on cadavers.

Interventions	**Outcome**			
	Success, n (%)	Failure, n (%)	Odds Ratio	p
Needle MINB	7(87.5)	1(12.5)	1	0.79
NFLJI MINB	10(83.3)	2(16.7)	1.40(95% CI, 0.11-18.6)	

6.5.6 Clinical safety issues of high-pressure NFLJI

A total of five participants (2 males and 3 females) with a median age of 23 (IQR 23-28) were included to evaluate the safety and feasibility of using NFLJI for MINB. This trial was stopped at five participants instead of six because one participant presented temporary nerve paresthesia following NFLJI anesthesia, creating a safety issue.

The recruitment took 3 weeks with a recruitment rate of 100% as the study was advertised on social media (*140*). No participants withdrew or reported concerns. Both NFLJI and needle MINB procedures were easily performed intraorally.

High-pressure NFLJIs achieved a preliminary success rate of 60%, whereas needle injections achieved a success rate of 100%. As for the clinical anesthesia effect (Table 6.2), the NFLJI group had a median (IQR) time to initial anesthesia of 1.4 (1.2- 1.9) min, onset time of 3.5 (2.9- 5.5) min, and duration of 252 (198- 276) min, whereas the needle group had 1.4 (0.6- 2.2) min time to initial anesthesia, 6.0 (4.8- 6.5) min onset time, and 182 (146-252) min duration. High-pressure NFLJIs resulted in median (IQR) pain scores of 3.0 (1.5- 4.3) and taste scores of 4.0 (3.3- 5.0), while the needle group showed median pain scores of 2.0 (1.0-4.0) and taste scores of 5.0 (5.0- 5.0) (Fig. 6.5 C- H).

In terms of complications (Fig. 6.5 I-M), needle injections caused 2 (40%) cases of bleeding, 0 (0%) cases of laceration, 2 (40%) cases of hematoma, 1 (20%) case of discomfort, and 0 (0%) cases of paresthesia. Meanwhile, high-pressure NFLJIs caused 0 (0%) cases of bleeding, 1 (20%) case of laceration, 2 (40%) cases of hematoma, and most importantly, 3 (60%) cases of discomfort, and 1 (20%) case of paresthesia. Among the 3 participants who had post-procedure discomfort, one had a hematoma (Fig. 6.5B), followed by nerve paresthesia at the left corner of the lower lip that lasted for two weeks; the other two had mild to moderate pain for three days when pressing the injection sites. Therefore, the pilot study was stopped.

Table 6.2 Demographic and clinical outcomes for the first pilot randomized clinical trial assessing the feasibility and safety of high-pressure NFLJI (620kPa,) and needle injections. Both interventions used 2 % Lidocaine with 1:100,000 epinephrine.

Demographic Outcomes		
Gender (Male/Total), n (%)	2 (40%)	
Age (year), median (IQR)	23 (23-28)	
Clinical Outcomes	**NFLJI (n=5)**	**Needle (n=5)**
MINB preliminary success rate, n (%)	3 (60%)	5 (100%)
Duration (min), median (IQR)	252 (198 - 276)	182 (146 - 252)
Time to initial anesthesia (min), median (IQR)	1.4 (1.2 - 1.9)	1.4 (0.6 - 2.2)
Onset of anesthesia (min), median (IQR)	3.5 (2.9-5.5)	6.0 (4.8-6.5)
Pain NRS difference, median (IQR)	3.0 (1.5-4.3)	2.0 (1.0- 4.0)
Taste score difference, median (IQR)	4.0 (3.3-5.0)	5.0 (5.0-5.0)
Bleeding, n (%)	0 (0%)	2 (40%)
Laceration, n (%)	1 (20%)	0 (0%)
Hematoma, n (%)	2 (40%)	2 (40%)
Post-procedure discomfort, n (%)	3 (60%)	1 (20%)
Paresthesia, n (%)	1 (20%)	0 (0%)

6.5.7 Clinical safety and feasibility of low-pressure NFLJI

The laboratory investigation revealed that low-pressure NFLJI (413 kPa) could achieve similar injection outcomes as high-pressure NFLJI (620 kPa) but with less risk of nerve damage since it created lower total work and maximum force on the soft tissue. Accordingly, another six participants (1 male and 5 females) were recruited for a second pilot RCT to evaluate the safety and feasibility of low-pressure NFLJI. Recruitment time and rate, and the withdrawal rate were the same as previous pilot RCT; no participants reported concerns. One participant was excluded before the procedure because of an unreported root canal treatment on the second premolar on the lower left region. A total of five participants (1 male, 4 female) with a median age of 23 (IQR 23-28) were included for analysis (Table 6.3).

MINBs using low-pressure NFLJI achieved a preliminary success rate of 60%, while MINBs using needle injections achieved a rate of 40%. Low-pressure NFLJIs showed a median (IQR) time to initial anesthesia of 0.8 (0.4-1.1) min, onset time of 4.2 (2.5-5.1) min, and duration of 171 (131-195) min, whereas needle injections had a median (IQR) time to initial anesthesia of 1.0 (0.5-1.4) min, onset time of 4.5 (3.7-4.9) min, and duration of 174 (126-219) (Fig 6.6A-D). As for the side effects, participants reported a median pain score of 0.8 (0.6-2.6), anxiety score of 0.9 (0.3-3.6), and taste score of 4.0 (3.5-5.0) with NFLJIs, and a median pain score of 1.8 (1.0-2.0), anxiety score of 0.7 (0.0-2.3) and taste score of 5.0 (5.0-5.0) with needle injections (Fig. 6.6 E-G).

Regarding the complications, the low-pressure NFLJIs induced 1 case of bleeding (20%), 1 case of laceration (20%), 1 case of hematoma (20%), 0 cases of discomfort and paresthesia (0%). The needle injections induced 0 cases of bleeding (0%) and laceration (0%), 1 case of hematoma (20%), 0 case of discomfort (0%) and paresthesia (0%) (Fig. 6.6 H-L). At the end of

the trial, participants were asked to choose their preference between the two techniques. Two participants preferred low-pressure NFLJI because the injection was fast and less painful. The other three participants preferred needle injection as they felt anxious about the novel NFLJI or disliked the noise of NFJLI.

Table 6.3: Demographic and clinical outcomes for the second pilot randomized clinical trial comparing the low-pressure NFLJI (413 kPa 1mL) with the needle injection (1mL). Both interventions used 2 % Lidocaine with 1:100,000 epinephrine.

Outcomes	**Low-pressure NFLJI (n=5)**	**Needle (n=5)**
Demographic Outcomes		
Gender (Male/Total), n (%)	1 (20%)	
Age, median (IQR)	23 (20-24)	
Clinical Outcomes		
MINB preliminary success rate, n (%)	3 (60%)	2 (40%)
	171 (131-195)	174 (126-219)
Duration (min), median (IQR)		
Time to initial anesthesia(min), median (IQR)	0.8 (0.4-1.1)	1.0 (0.5-1.4)
Onset of anesthesia(min), median (IQR)	4.2 (2.5-5.1)	4.5 (3.7-4.9)
Pain VAS difference, median (IQR)	0.8 (0.6-2.6)	1.8 (1.0-2.0)
Anxiety VAS difference, median (IQR)	0.9 (0.3-3.6)	0.7 (0.0-2.3)
Taste score, median (IQR)	4.0 (3.5-5.0)	5.0 (5.0-5.0)
Bleeding, n (%)	1 (20%)	0 (0%)
Laceration, n (%)	1 (20%)	0 (0%)
Hematoma, n (%)	1 (20%)	1 (20%)
Post-procedure discomfort, n (%)	0 (0%)	0 (0%)
Paresthesia, n (%)	0 (0%)	0 (0%)

6.6 Discussion

This study advanced the understanding of how NFLJI parameters affect its penetration in soft tissues and the risks associated with tissue damage. In addition, an optimal NFLJI technique was developed for MINBs based on *in vitro*, *ex vivo*, and clinical studies. The optimized low-pressure NFLJI technique achieved effective anesthesia while reducing the risk of tissue damage.

6.6.1 The liquid jet momentum

Our *in vitro* experiments showed that the NFLJI total work and maximum force were directly proportional to NFLJI supply pressure. The increased supply pressure resulted in increased total linear momentum of the liquid jet. This momentum could determine both the penetration depth and the risk of tissue damage. Consequently, the estimated jet pressure upon impingement and penetration is directly proportional to NFLJI supply pressure. This finding can explain why high-pressure NFLJIs showed a high risk of post-operative discomfort and nerve injury while low-pressure NFLJIs had none of these cases.

6.6.2 A predictive model for penetration depth

Previous studies indicated that NFLJI dispersion and penetration depend on the injector parameters, such as supply pressure, volume, and orifice diameter (*63*); operative parameters, such as standoff distance and loading pressure (*181*); Young's modulus of tissue (*64*); and the viscosity and density of the injected fluid (*65*). Among these factors, the property of fluid and tissue of the injection site cannot be changed; the operative parameters of NFLJI are predefined using minimal standoff distance and a loading force of 0.3N for our clinical trial. Therefore, only the injector parameters can be adjusted to optimize the injection outcome. Our study found that NFLJI penetration depth is directly correlated to pressure and volume. These observations are in agreement with previous studies conducted on ballistic gelatin (10% w.t) (*188*) and cadaver skin

(*65*), using NFLJI with a volume ranging from 0.2 to 2.5 mL and with a supply pressure ranging from 600 kPa to 20MPa (*65, 188*).

In a previous study (*64*), a predictive model was created based on the liquid jet velocity, the nozzle diameter, the tissue's Young's modulus, and the fluid density in the scenario with or without backflow. This model assumed that the flow behaved as a confined jet in a closed tube, implying that the jet center-line velocity decreases approximately linearly with distance. However, based on the high-speed video of jet penetration and time history, the jet velocity reduction was not linear (Fig. S6.2A). The observed jet flow is an impulsive jet with a vortex head. The observations are not consistent with the hypothesis of a confined jet flow.

Nevertheless, this model Eq (2) (*64*) was used with our data to predict the penetration depth based on the jet velocity measured from the high-speed video and compare it with the measured penetration depth, i.e.

$$\frac{v_m}{v_0} = m\left(\frac{x}{D_0}\right) + b \quad , \qquad \text{Eq (10)}$$

where v_m is the critical center-line velocity required to induce failure, v_0 is jet exit velocity, x is the jet travel distance, and D_0 is the nozzle diameter. The m and b were calculated by attempting a linear regression of the data, which obeyed a non-linear trend.

The predicted penetration depth compared to the real penetration depth showed a high root-mean-square deviation (RSMD) of 54.2 mm (*189*), calculated as:

$$RSMD = \sqrt{\frac{\sum(\hat{y}-y)^2}{n}} \qquad \text{Eq (11)}$$

Where the $\hat{y}$ is the estimated depth, and y is the actual depth obtained from *in vitro* experiment. The previous model (*64*), which assumes a linear reduction of jet velocity, could only fit 7.8-20.5% of the observed data (Table S6.2, Fig. S.2B). Therefore, a better model assuming non-linear jet velocity reduction in the tissue is desirable to obtain an accurate depth prediction.

6.6.3 Mental incisive nerve blocks

In our cadaver and clinical studies, MINB anesthesia with NFLJIs had similar success rate to that achieved with needle injections. This study is the first conducted to assess the use of NFLJI for MINB in either cadavers or clinical practices.

The previous literature on MINB was limited to needle injections only and reported success rates ranging from 50% to 93.8% with lidocaine (Table 6.4) (*179*) (*38, 151, 190-192*). This range falls within the success rates obtained with NFLJI and needle injection in cadavers and clinical trials.

The success rate of MINB could be improved by increasing the volume (*31*) or the potency of the anesthetic (*12, 191*). However, high potency is also correlated with high tissue toxicity and higher risk of nerve paresthesia, especially for mandibular nerve blocks (*160*). Hence, 2% lidocaine is recommended for patients' safety.

Table 6.4 The clinical efficacy of MINB in previous clinical trials.

Reference	Anesthetic	Injection volume	Success cases	Sample size	Efficacy
(*179*)	4% articaine with 1:100,000 epinephrine	1.8 mL	30	32	93.8%
(*191*)	4% articaine with 1:100,000 epinephrine	0.6 mL	32	40	80%
	2% lidocaine with 1:100,000 epinephrine	0.6 mL	28	40	70%
(*38*)	2% lidocaine with 1:200,000 epinephrine	2.0 mL	27	51	53%
(*190*)	2% lidocaine with 1:80,000 epinephrine	2.2 mL	33	38	86.8%
(*192*)	2% lidocaine with 1:100,000 epinephrine	0.9 mL	30	41	73%
(*151*)	2% lidocaine with 1:80,000 epinephrine	2 mL	30	38	78.9%
Total events	4% articaine with epinephrine	<1 mL	32	40	80%
		>1 mL	30	32	93.8%
		All	62	72	86.1%
	2% lidocaine with epinephrine	<1 mL	58	81	71.6%
		>1 mL	90	127	70.8%
		All	148	208	71.2%

6.6.4 Complications of NFLJI nerve blocks

In our study, the pressure created by both NFLJIs and needle injections had the potential to injure nerves or blood vessels, leading to paresthesia, hematoma, or discomfort. However, high-pressure NFLJI was more likely to cause these damages because it induced a significantly higher estimated jet impingement pressure and maximum jet penetration pressure compared to those of low-pressure NFLJI and needle injection (Fig. 6.4 B C).

One case of mental nerve paresthesia was reported during the first pilot trial using high-pressure NFLJI (620 kPa). Paresthesia is a common complication in which patients present persistent anesthesia or altered sensation beyond the expected duration of anesthesia that can last from days to months (*12*). It is usually caused by trauma to the mental nerve or by the pressure from bleeding and hematoma (*12*). In our study, the patient first presented a significant hematoma (Fig. 6.4B) at the mental foramen region after injection before reporting the paresthesia.

Besides a case of paresthesia, high-pressure (620kPa) NFLJI caused more hematoma (40%) and discomfort (60%) than low-pressure NFLJI, indicating more tissue damage. The low-pressure NFLJI (413kPa) group caused no discomfort or paresthesia and resulted in only one incident of hematoma (20%). As shown in our in vitro experiment, this result is probably because low-pressure NFLJIs produce less total work and maximum force in the tissue than high-pressure NFLJIs, therefore causing more minor tissue damage.

In addition, one case of laceration was reported with both high- and low-pressure NFLJIs (Fig. S3 E). Our group has previously shown that lacerations were probably caused by jet regurgitation and backflow when the jet impacts hard tissue during perpendicular injection (Fig. S3 F), which can be minimized by employing an oblique injection technique (*140*). Even though

the oblique technique was used, there was still a risk of laceration (*140*). Further studies are therefore needed to eliminate the laceration risk. Other reasons for laceration could be patients' head movement and operators' hand movements during the injection, as reported in our previous study(*140*), or a sharp edge at the nozzle tip.

6.6.5 The estimated pressure during injection

To explain why lower-pressure NFLJI is safer than high-pressure NFLJI, understanding the jet pressure when penetrating soft tissue is necessary. For example, the required pressure for a liquid jet to pierce through human skin is 690kPa (*193*), whereas the pressure at which the nerve damage occurs is 80kPa (*187*). Therefore, jet pressure must initially be high enough to pierce the skin while delivering drugs within the tissue at a low pressure to prevent nerve damage.

The NFLJI system uses pneumatic pressure to drive a that impacts the liquid to create the jet. The supply pressure of the system, the pressure when the liquid jet exits the nozzle, and the pressure when the jet travels inside the tissue are different due to the energy loss and area difference between the nozzle orifice and wound. Therefore, the pressure generated by the jet when it travels inside soft tissue should be estimated by dividing the instantaneous force measured using the force transducer by the instantaneous area of the jet measured using the high-speed video record.

Our study discovered that both high- and low-pressure NFLJI created imping pressure higher than 690 kPa (*193*) to pierce through the skin. However, low-pressure NFLJI can keep a penetration pressure beneath 80kPa (*187*) to avoid nerve damage. In contrast, the high-pressure NFLJIs could create a penetration pressure higher than 80 kPa, which increased the risk of nerve damage when jet traveling inside soft tissue.

This observation would explain the nerve paresthesia case that occurred with high-pressure NFLJI in the pilot RCT. It also provided clinical guidance to dentists for selecting proper injector parameters to minimize complications while maintaining the anesthesia outcome.

6.6.6 Injection pain and pressure

The low-pressure NFLJIs showed a trend of lower pain scores than those of the high-pressure NFLJI group. This trend is presumably because the low-pressure NFLJI caused lower maximum force, total work (Fig. 6.3 C&D), and maximum penetration pressure (Fig. 6.4C), hence less mechanical pain stimulus on soft tissue. The relationship between NFLJI pressure and pain feelings warrants further investigation.

This mechanical pain stimulus theory can be supported by two clinical studies investigating needle injection speed and pain feeling. Slow injections (2mL/min) create significantly lower pain scores than rapid injection (8mL/min) on patients receiving mandibular nerve blocks (*152*) or MINBs (*194*). Similarly, our *in vitro* experiment for needles demonstrated that slow injection (1.8 ml/min) created lower total work than rapid injection (7.2mL/min) (Fig. 6.3D), hence less mechanical pain stimulus on soft tissue.

A slow-speed needle injection (1.8 mL/min) was used for the needle injection group in our study for patients' comfort. This slow injection gave a relatively lower pain score in the study, making it more challenging to see the difference in pain score between the NFLJIs and the needle injections.

6.6.7 Strengths, limitations, and future directions

Gelatin (5 wt.%) is an acceptable phantom for injection experiments because it has Young's modulus similar to that of oral soft tissue. However, its fracture toughness is significantly lower than that of soft tissue. In addition, when dispersing in gelatin, the vortex jet flow creates a crack by shear force since gelatin is a non-porous material. Meanwhile, when dispersing in soft tissue, the jet diffuses through the porous structure instead of creating a crack. This results in smaller wound size and lower regurgitation volume. Therefore, a porous phantom material with more realistic properties would be desirable for future research.

Our study presented a jet central core velocity based on the momentum force and time as well as the volume and fluid dentistry, this velocity cannot represent the jet velocity when it exits the orifice. Jet exiting velocity could be calculated by a few methods, for example the piston speed can be related to the volumetric average jet speed (*195*), the momentum force, fluid density and aera of orifice can provide the jet speed when jet impinging on a force sensor (*195*) (*196*). Though this paper focused on the supply pressure of NFLJI and the correlated risk, further studies are needed make an link between NFLJI parameter, jet dynamic velocity and outcomes.

Our study showed that it is feasible to conduct an RCT with relative safety using low-pressure NFLJI. In addition, the recruitment rate was high if social media was used. Future trials should consider recruiting patients who visit the dental clinic for tooth extraction or filling to get more samples.

Safety is the biggest concern before conducting a formal RCT using NFLJI. Our study presented a pressure estimation to assess the risk of nerve injury and reduced the risk of nerve paresthesia by reducing the injector's supply pressure. However, the injection force was

measured using a force transducer, which is a net force including the jet penetration or needle injection force, the gravity force of the liquid, and the friction from tissue phantoms. Therefore, the estimated injection pressure might be slightly overestimated than the real value. A further force calculation considering the type of forces mentioned above would be desirable. In addition, more studies are still needed to minimize the other complications of NFLJI, such as mucosa laceration. As volume and potency influence the anesthesia efficacy, future studies should consider increasing the volume from 1mL to 1.8mL since 2% lidocaine has lower potency and efficacy than other anesthetics.

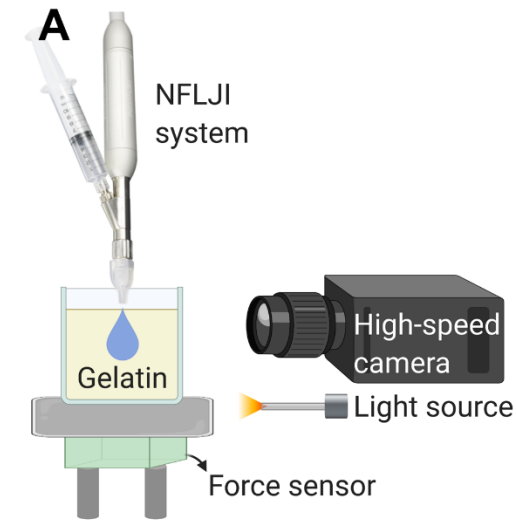
Cadaver experiments and pilot RCTs both indicated that the efficacy of NFLJI is comparable to that of needle injection for MINB. However, with only a total of ten cadavers and ten human subjects in this study, the limited sample size could not ensure strong statistical power to claim non-inferiority in the efficacy of NFLJIs compared to needle injections. A non-inferiority randomized controlled trial using a cross-over design could have sufficient power with 160 to 492 participants based on statistical simulation (α= 0.05, β= 0.2) (*174*). Future studies should report the efficacy of NFLJI and needle anesthesia, the odds ratio, and the frequencies of concordant-discordant results per group. They should run the statistical analysis using a mixed model logistic regression.

6.7 Conclusion

Pneumatic NFLJI penetrates the oral soft tissue deep enough to effectively deliver anesthetic around the mental nerve foramen. Low-pressure NFLJI is relatively safer than high-pressure NFLJI because the former showed the lower value of maximum force and total work similar to those of needle injection and lower value of estimated jet impingement pressure and maximum jet penetration pressure. Therefore, reducing NFLJI supply pressure can help

minimize its complications while still achieving clinical outcomes comparable to needle injections.

On cadavers, the simulated success rates of MINB were 83.3% in the NFLJI group and 87.5% in the needle group. The preliminary clinical success rates of MINB were 60% in NFLJI and 70% in the needle group.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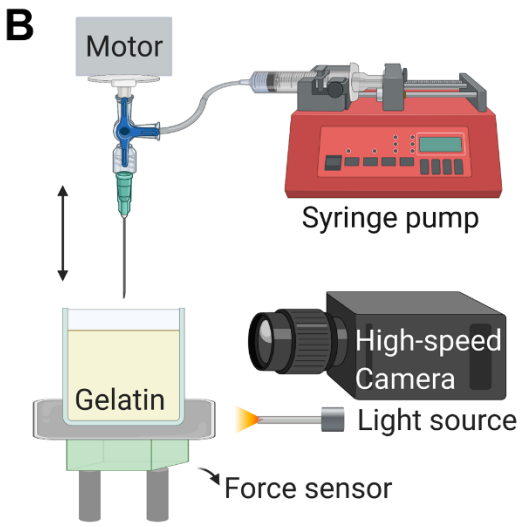
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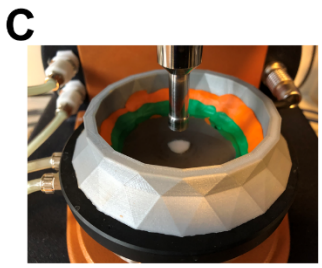
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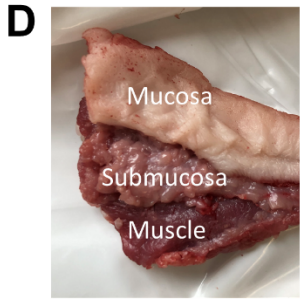
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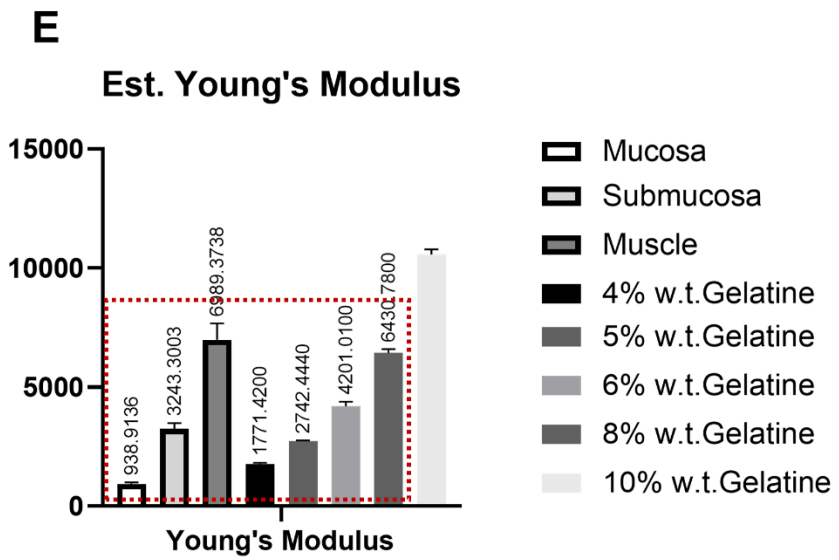
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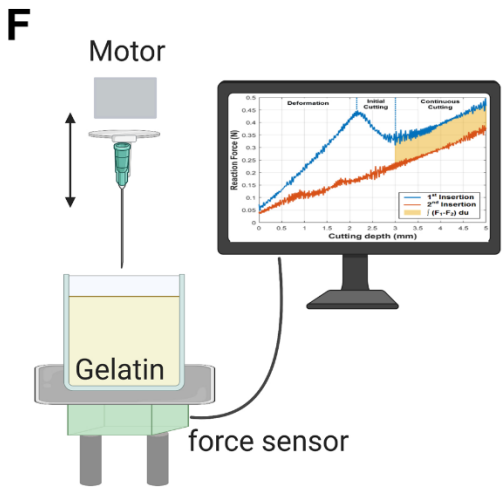
5% w.t.Gelatine
6% w.t.Gelatine
8% w.t.Gelatine
10% w.t.Gelatine


F
Motor
Gelatin
force sensor

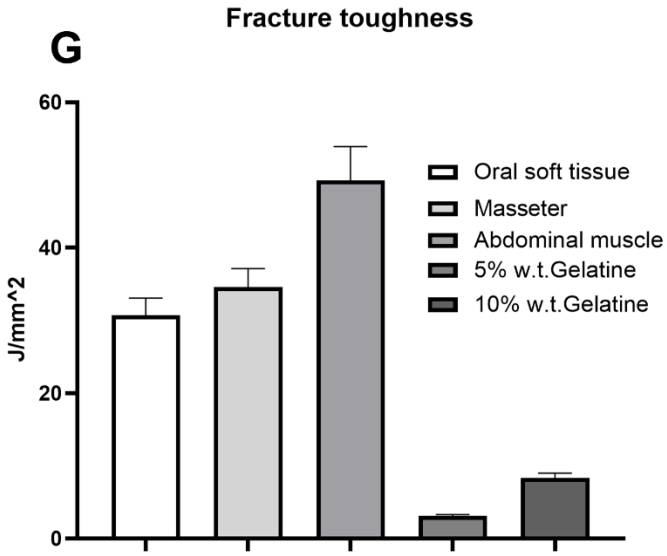

G
Fracture toughness
60
40
20
0
J/mm^2
Oral soft tissue
Masseter
Abdominal muscle
5% w.t.Gelatine
10% w.t.Gelatine

Figure 6.2 (A) Experimental set up for in vitro needle injection and (B)NFLJI.(C) Measurement of Young's modulus for (D) oral soft tissue and phantom materials, 4-10% wt. gelatin. (E) Young's modulus of 5% gelatin is within the range of oral soft tissue, while 10% gelatin is stiffer than oral soft tissue. (F) Concept of fracture toughness measurement using needle piecing method. (G) Fracture toughness of oral soft tissue is higher than that of 5% gelatin. A, B, and F were created with BioRender.

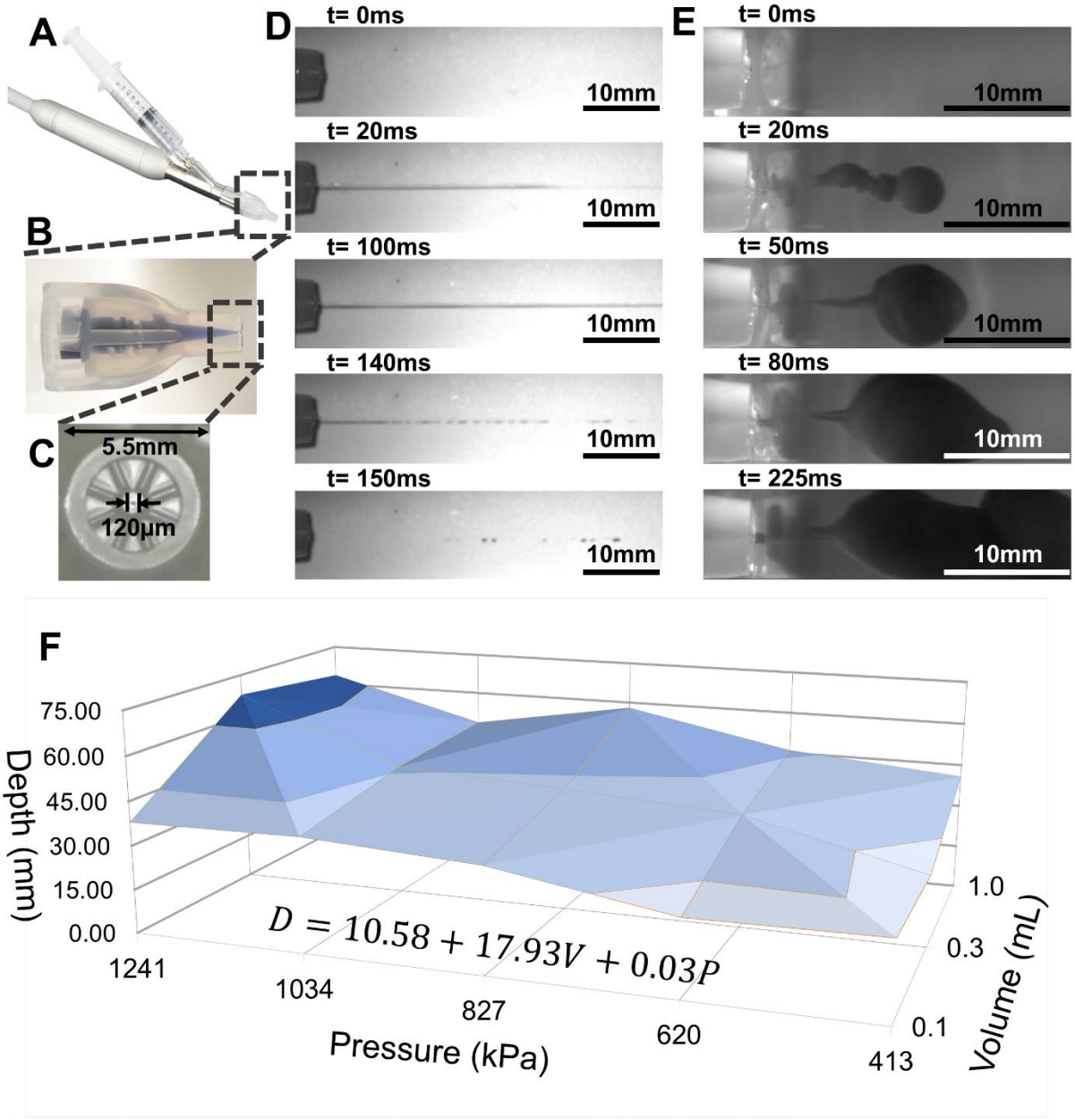

A
B
C
5.5mm
120μm
D
t= 0ms
10mm
t= 20ms
10mm
t= 100ms
10mm
t= 140ms
10mm
t= 150ms
10mm
E
t= 0ms
10mm
t= 20ms
10mm
t= 50ms
10mm
t= 80ms
10mm
t= 225ms
10mm
F
75.00
60.00
45.00
30.00
15.00
0.00
Depth (mm)
1241
1034
827
620
413
Pressure (kPa)
1.0
0.3
0.1
Volume (mL)
D = 10.58 + 17.93V + 0.03P
G
H
I
J
K
L
M
MINB on cadaver
Success Rate (100%)
100
80
60
40
20
0
83.3
87.5
NFLJI
Needle

Figure 6.3 (A) Needle-free liquid jet injection system in this study, view from (B) side and nozzle tip (C). The injection dispersion in (D) air and in (E) 10% gelatin. (F)The penetration depth increased with supply pressure and injected volume. (G)The MINB using needle, example of (H) successful and (I) failed injection result after dissection. (J) The MINB using NFLJI, examples of (K) successful and (L)failed injection result. (M) The simulated success rate of MINB on cadaver.

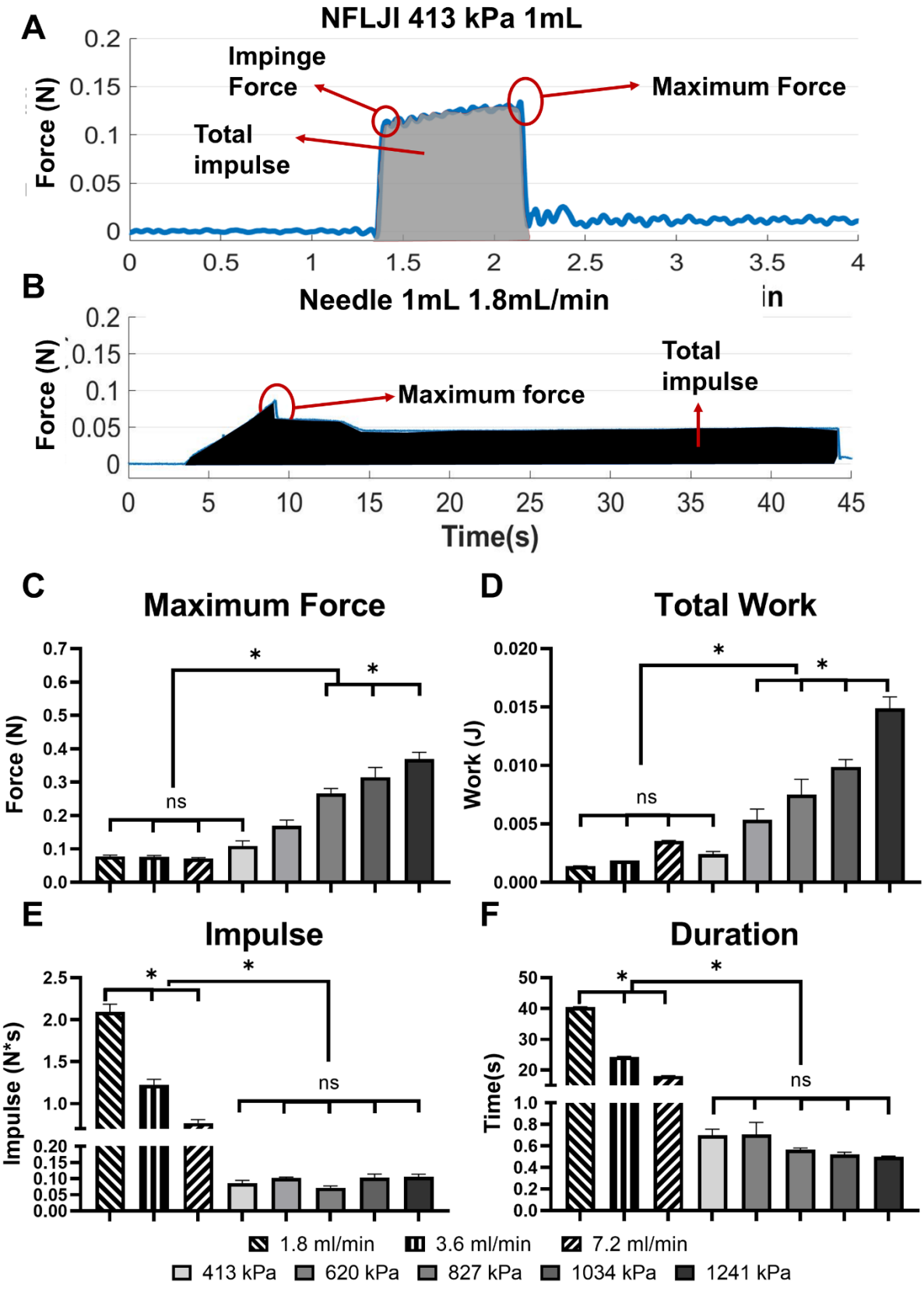

A
NFLJI 413 kPa 1mL
Impinge Force
Maximum Force
Total impulse
Force (N)
B
Needle 1mL 1.8mL/min
Maximum force
Total impulse
Force (N)
Time(s)
C
Maximum Force
Force (N)
ns
D
Total Work
Work (J)
ns
E
Impulse
Impulse (N*s)
ns
F
Duration
Time(s)
ns
1.8 ml/min
3.6 ml/min
7.2 ml/min
413 kPa
620 kPa
827 kPa
1034 kPa
1241 kPa

Figure 6.4 (A) The force signal versus time of NFLJI using 413 kPa and 1 mL. (B) The force signal of needle injection using 1mL with insertion speed of 5mm/s and injection flow rate of 1.8mL/min. (C -F) The maximum force, total work, impulse, and duration of injections using needle with 1.8, 3.6, and 7.2 mL/min flow rate and using NFLJI with 413 to 1241 kPa supply pressure.

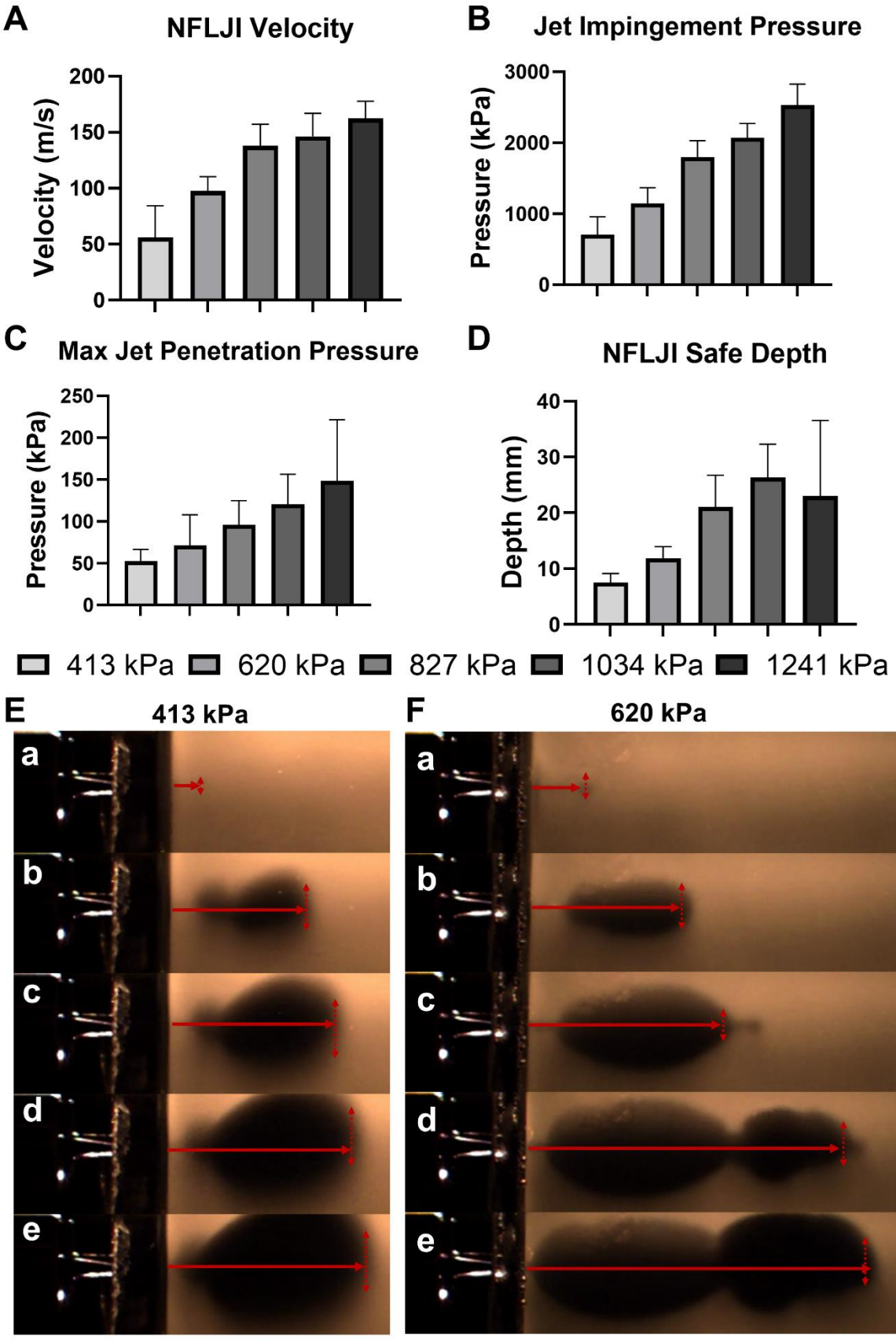

A
NFLJI Velocity
Velocity (m/s)
200
150
100
50
0
B
Jet Impingement Pressure
Pressure (kPa)
3000
2000
1000
0
C
Max Jet Penetration Pressure
Pressure (kPa)
250
200
150
100
50
0
D
NFLJI Safe Depth
Depth (mm)
40
30
20
10
0
413 kPa
620 kPa
827 kPa
1034 kPa
1241 kPa
E
413 kPa
F
620 kPa
a
b
c
d
e

Figure 6.5 (A-D) Analysis of NFLJI based on the force - time history and depth- time history. (E-F) the high-speed video record showed that jet penetration depth versus time.

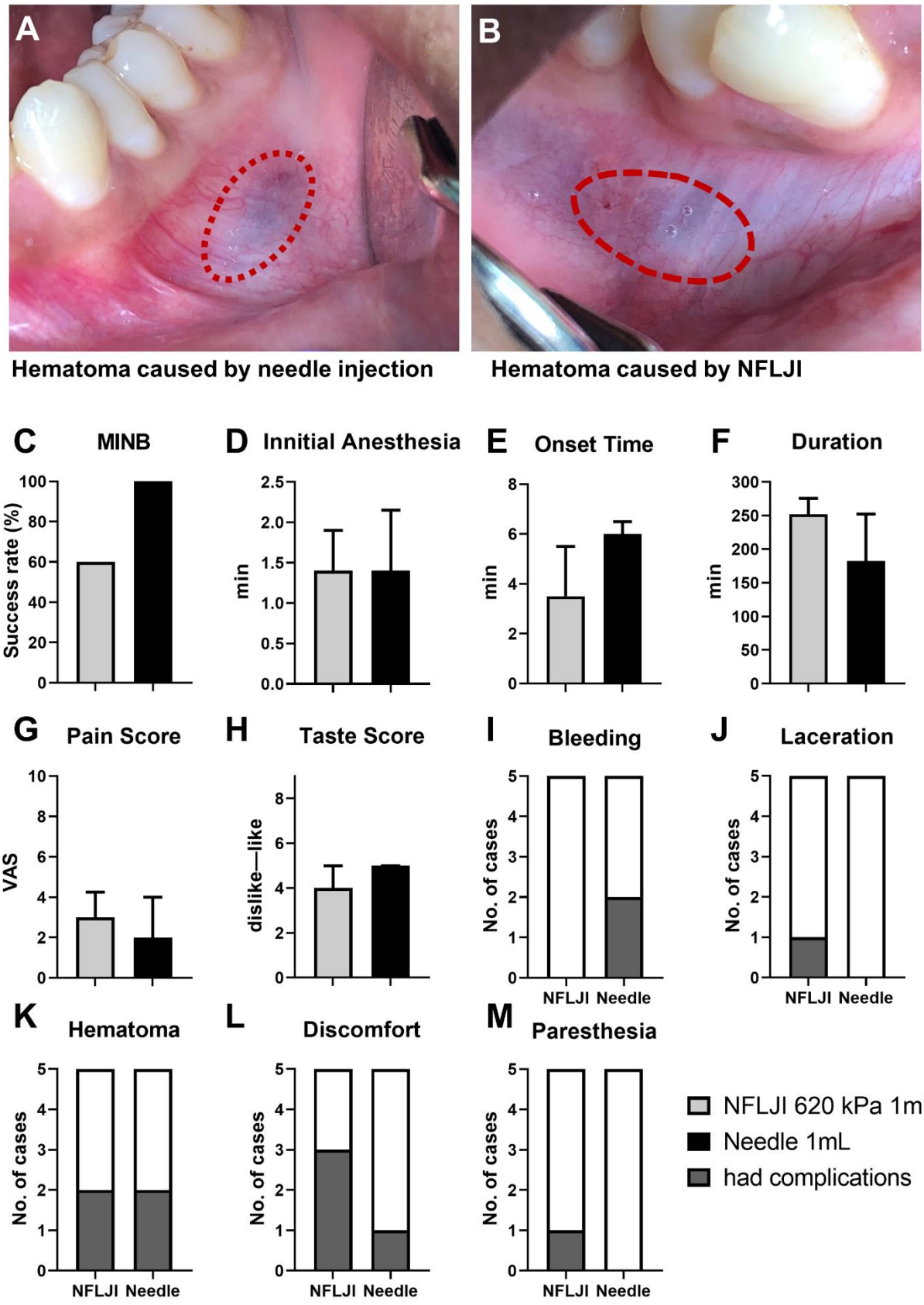

A
B
Hematoma caused by needle injection
Hematoma caused by NFLJI
C MINB
Success rate (%)
D Innitial Anesthesia
min
E Onset Time
min
F Duration
min
G Pain Score
VAS
H Taste Score
dislike—like
I Bleeding
No. of cases
J Laceration
No. of cases
NFLJI Needle
K Hematoma
No. of cases
L Discomfort
No. of cases
M Paresthesia
No. of cases
NFLJI Needle
NFLJI 620 kPa 1mL
Needle 1mL
had complications

Figure 6.6 Cases of hematoma cause by (A) needle insertion and (B) NFLJI. (C-M). Clinical outcome of first pilot study comparing MINB using needle or NFLJI.

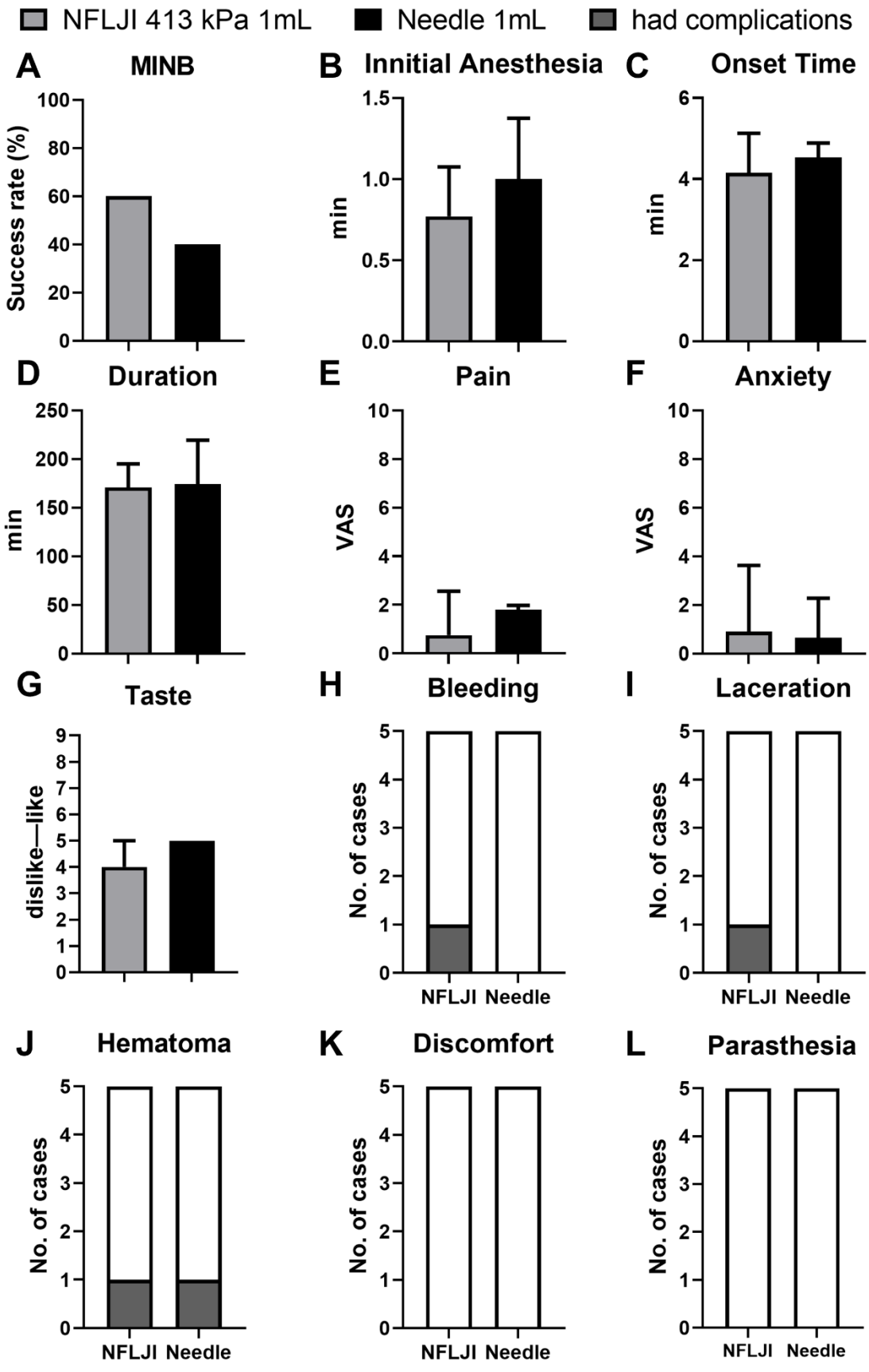

NFLJI 413 kPa 1mL
Needle 1mL
had complications
A
MINB
Success rate (%)
B
Innitial Anesthesia
min
C
Onset Time
min
D
Duration
min
E
Pain
VAS
F
Anxiety
VAS
G
Taste
dislike—like
H
Bleeding
No. of cases
I
Laceration
No. of cases
J
Hematoma
No. of cases
K
Discomfort
No. of cases
L
Parasthesia
No. of cases
NFLJI
Needle

Figure 6.7 The second pilot RCT to validate the safety of refined NFLJI (n=5). There was a significant improvement of post-procedure discomfort in the refined NFLJI group compared to the first pilot study, and no paresthesia occurred.

Acknowledgments: We sincerely thank Robert L'Heureux B.S., Jamie Brisebois B.S. from Department of cell and anatomy, McGill University, for organizing the cadaver experiments. We also appreciate the generosity of the body donors and their families. Our gratitude further extends to Nathalie Morin DDS, Rosa Menale DA, Ann Marie Plante DA from Faculty of Dentistry, McGill University for organizing the clinical trials, Dr. Guangyu Bao for the rheometer training, and Ms. Joan O'Malley for the photo studio.

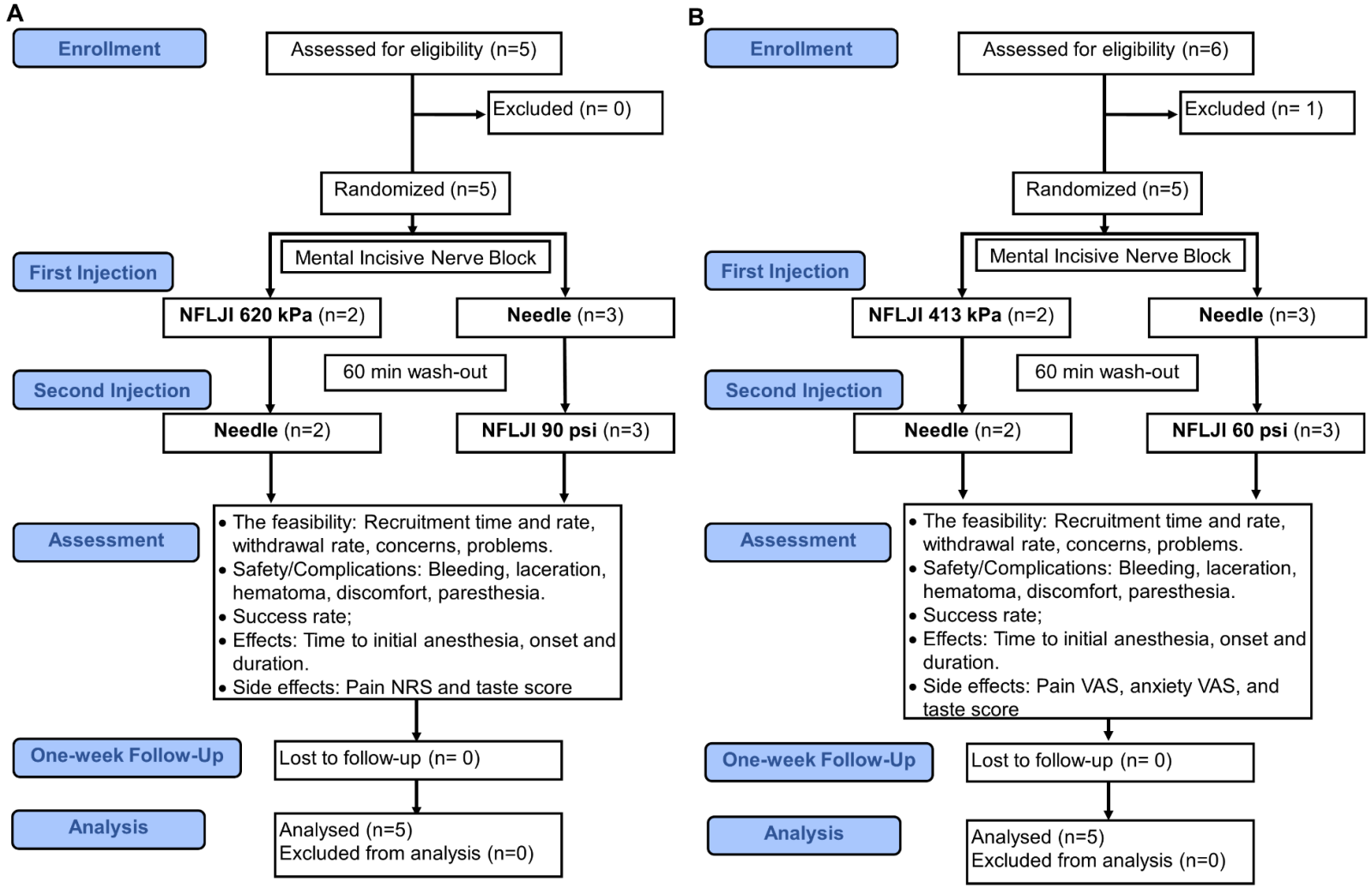
Funding: This research was sponsored by Natural Sciences and Engineering Research Council of Canada (543972-19 and 366077487). The first author is sponsored by the Clifford C.F. Wong Fellowship, réseau de Recherche en Santé Buccodentaire et Osseuse and Alpha-Omega Foundation of Canada from McGill University and the Doctoral fellowship from the Chinese Scholarship Council.

Author contributions: Q. GAO contributed to the conception, study design, data acquisition, and interpretation of the study, drafted and revised the manuscript. D. TAQI, M. ABUSAMAK, K. MENASSA, Z. He, A. HENLEY, S. Groen, and R. TAYHE contributed to the experimental design, data acquisition, and critically revised the manuscript. G. NOEL and Z. D. KHATCHADOURIAN, A. VELLY, E. ELHAM, L. MONGEAU and F. TAMIMI contributed variously to the conception, design, data interpretation, and revision of the study. All authors gave their final approval and agreed to be accountable for all aspects of the work. The authors declare that there is no conflict of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

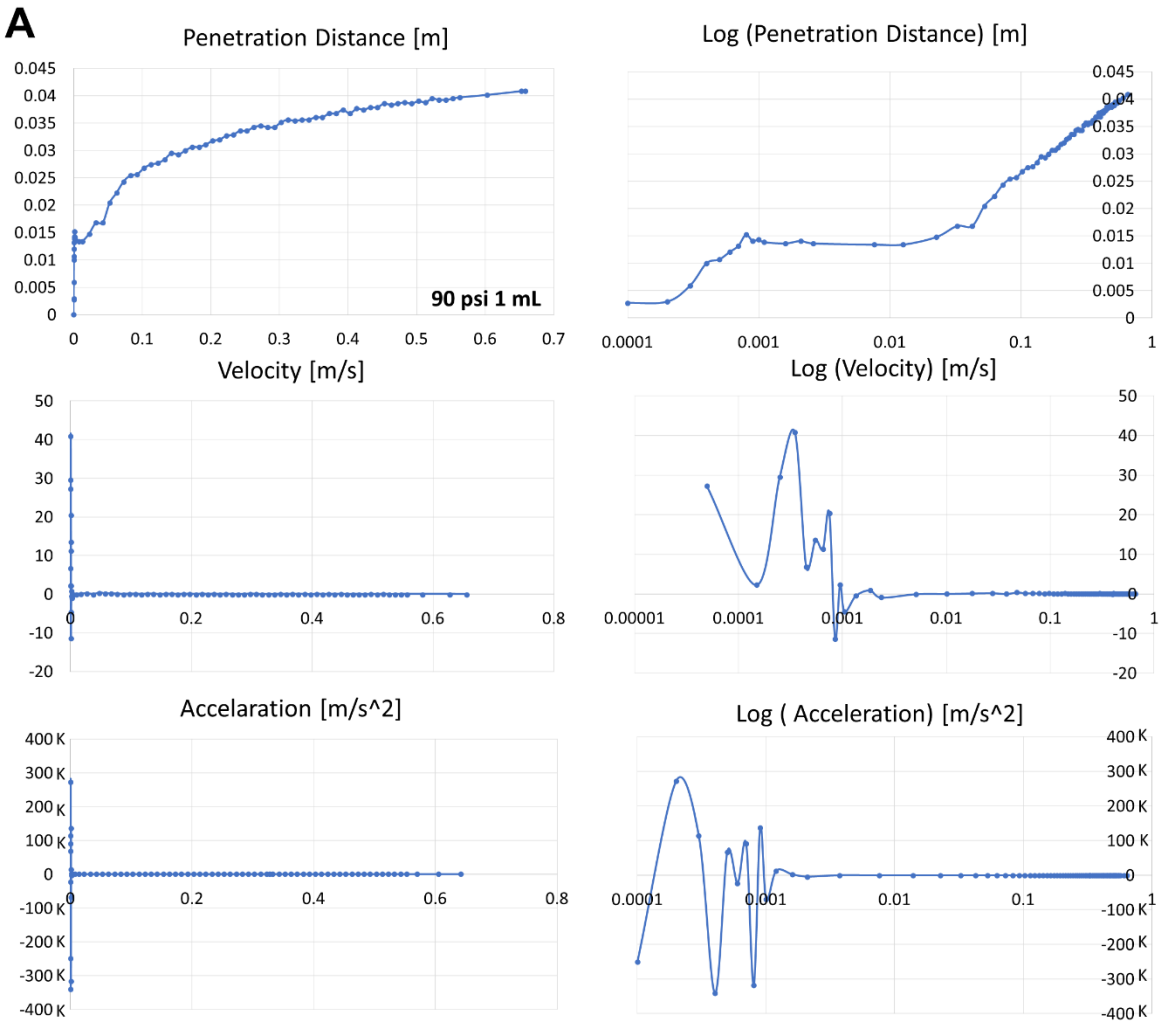
Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Mr. Karim Mennasa is the founder of Medial International Technology

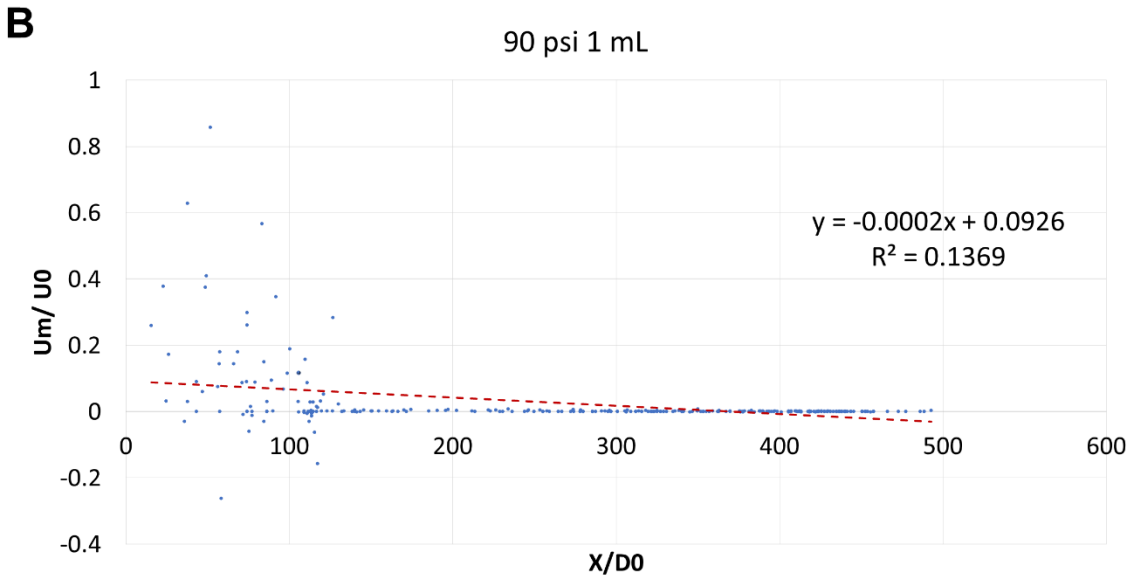
Canada Inc. and invented the needle-free liquid jet injection system in this study. He has no financial contribution to the study.

Supplementary Figures and Tables

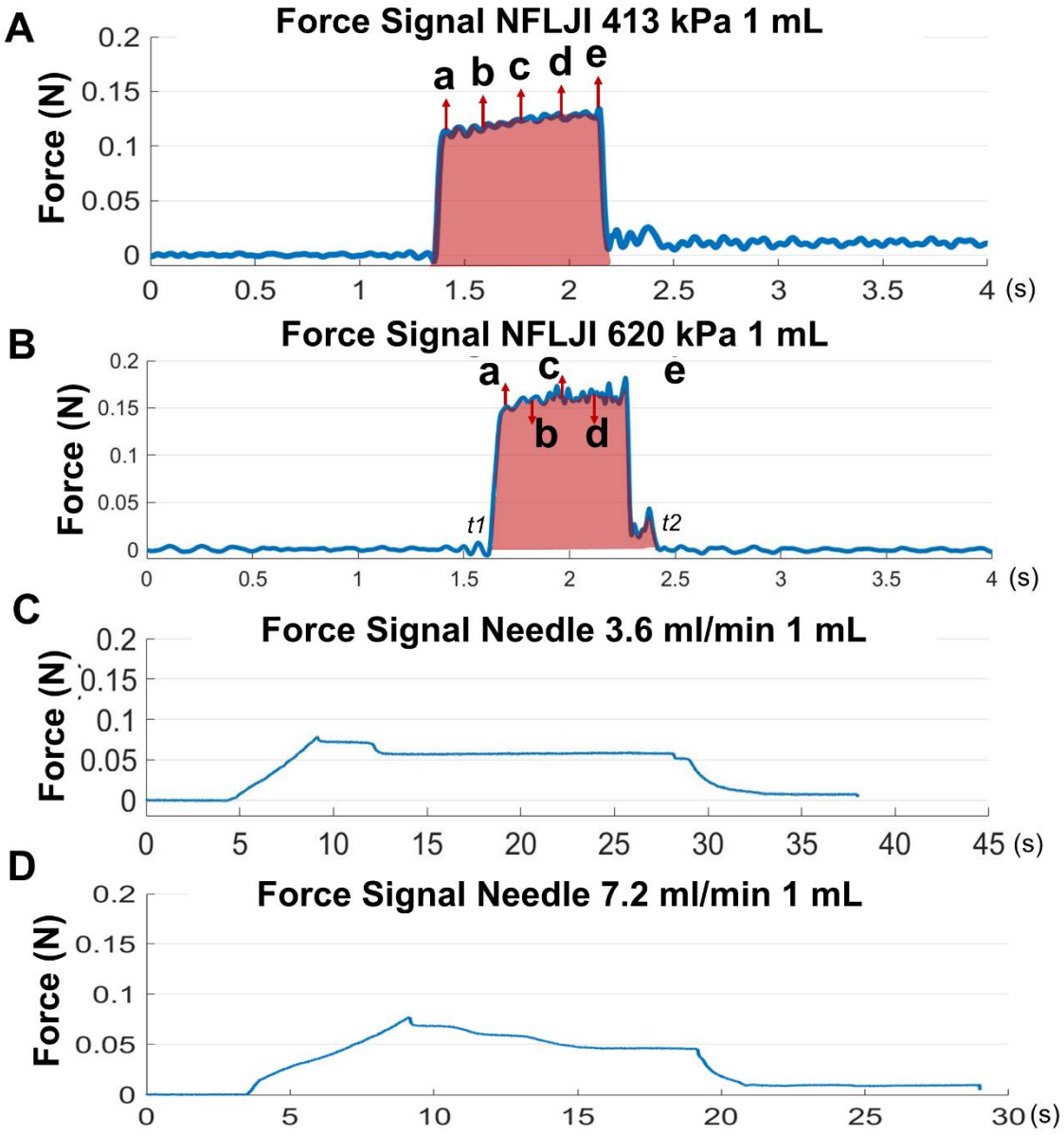


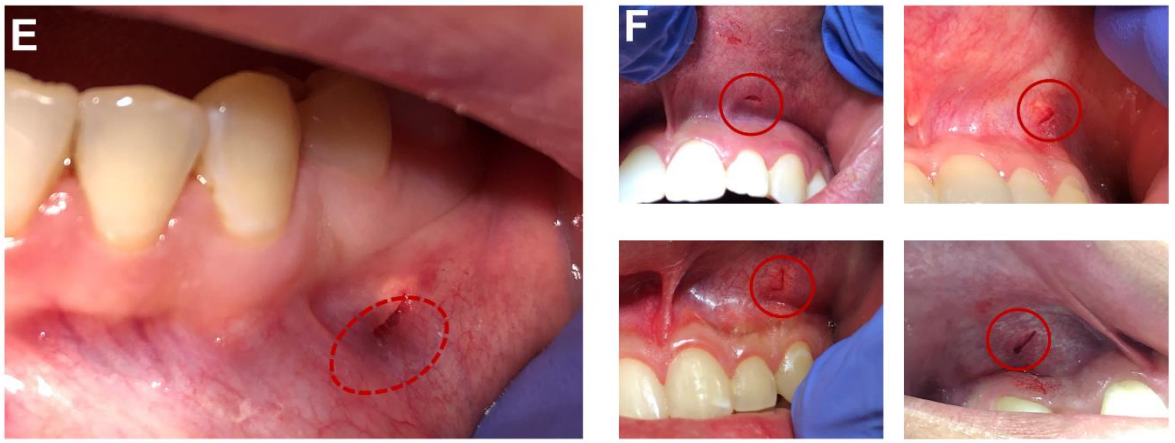
Supplementary Figure S6.1 (A-B) the CONSORT flow chart of two pilot randomized clinical trials.


A
Penetration Distance [m]
90 psi 1 mL
Log (Penetration Distance) [m]
Velocity [m/s]
Log (Velocity) [m/s]
Accelaration [m/s^2]
Log (Acceleration) [m/s^2]


B
90 psi 1 mL
y = -0.0002x + 0.0926
R² = 0.1369
Um/ U0
X/D0

Supplementary Figure S6.2 (A) Example of NLFJI using 90 psi and 1mL, the penetration distance is measured frame by frame from the high-speed camera video, the velocity and acceleration versus time were calculated accordingly. The velocity change was not linear. (B) Model fitting based on a previous study (*64*) using the example of 90 psi and 1 mL, another model fitting is shown in table S1.

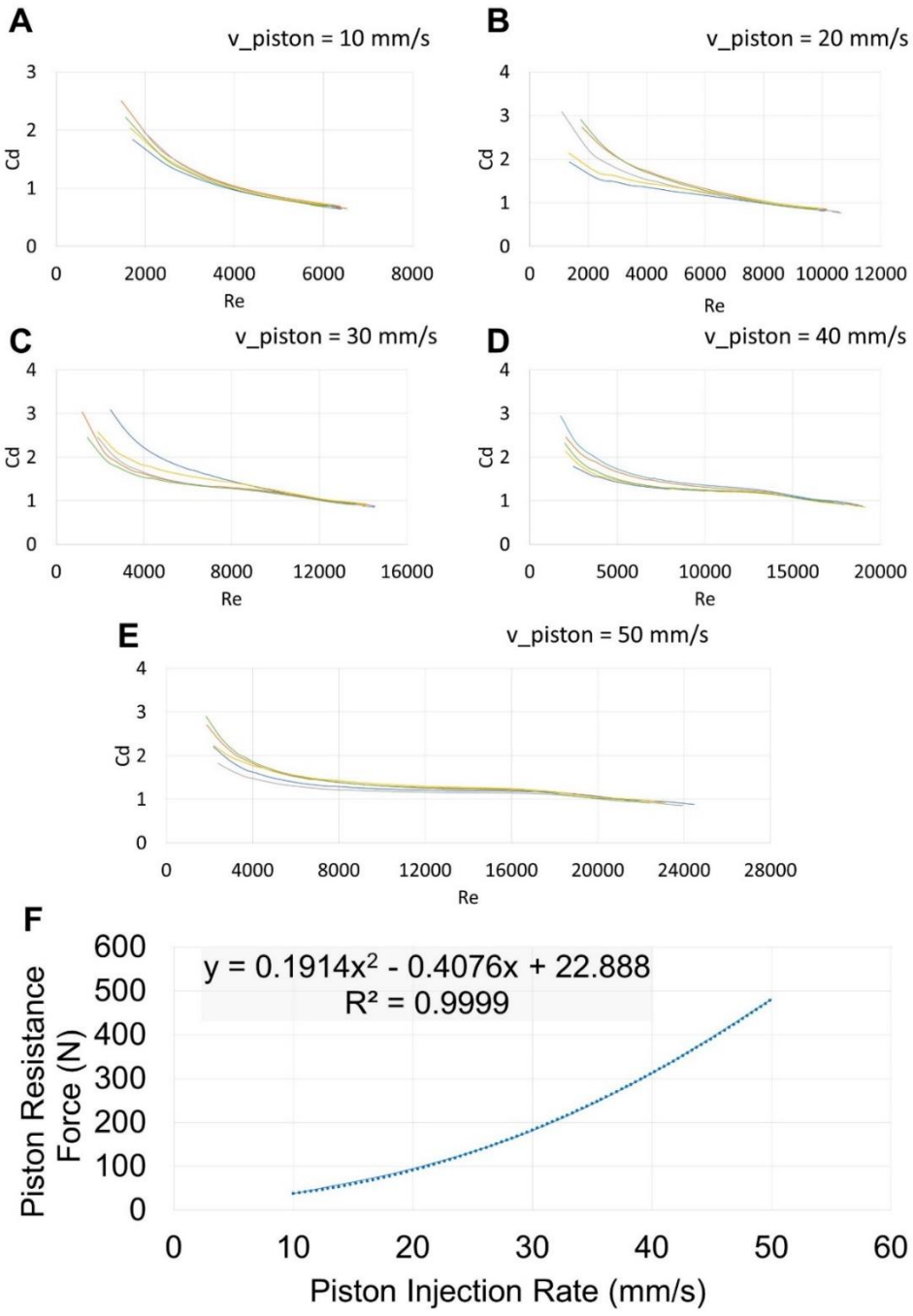




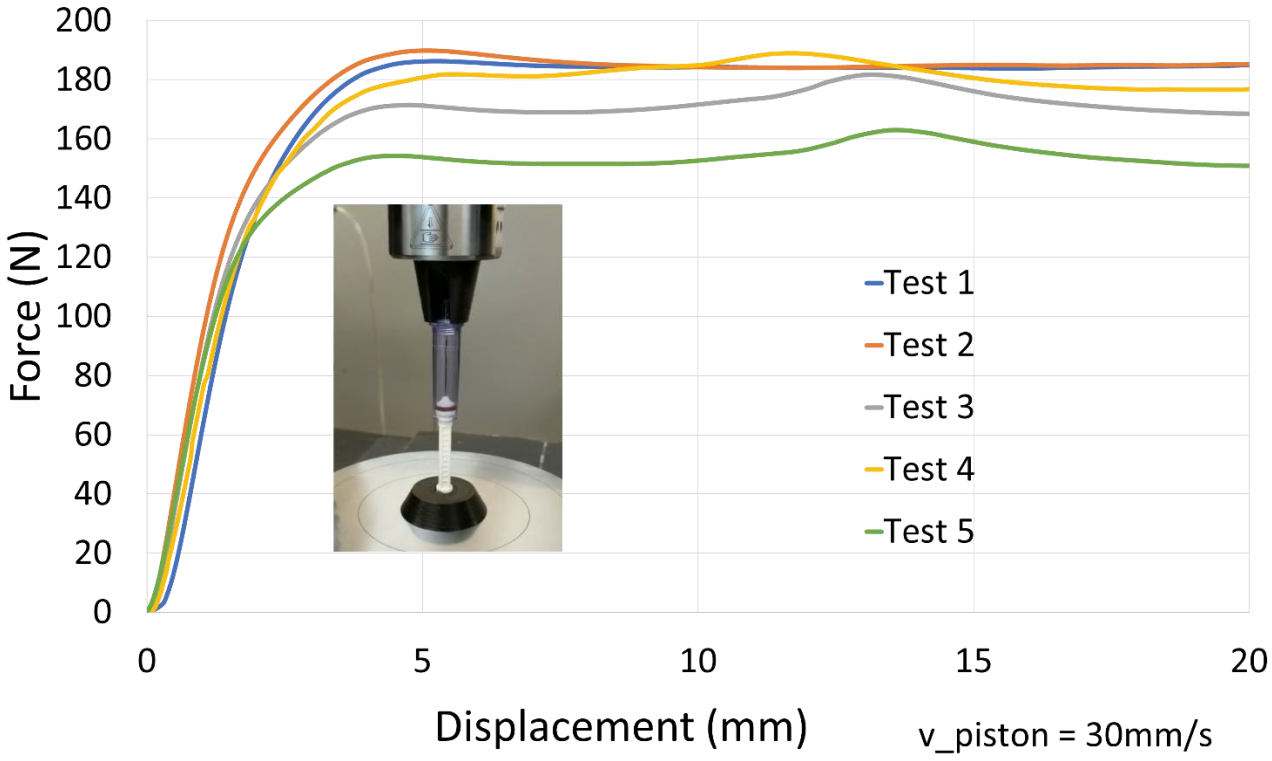
Laceration, NFLJI mental nerve block

Laceration, NFLJI infiltration anesthesia

Supplementary Figure S6.3 (A-D) Examples of force-time history for NFLJI and needle injections. The a-e in Fig. A and B are matched with a-e in Fig. 4 E and F. (E-F). Examples of laceration caused by NFLJI for mental nerve block and a few cases caused by perpendicular infiltration anesthesia.



Supplementary Figure S6.4 A-E the relationship between discharge coefficient and Reynolds numbers based on different piston injection rate. **F** Piston resistance force at different piston injection rates.



Supplementary Figure S6.5 Methodology for collecting piston displacement and piston resistant force, these data were used to calculate the discharge coefficient and Reynold number.

Supplementary Table 6.1 Continuation of Figure 4, showing examples of estimation for jet impinge pressure (first raw) and jet maximum penetration pressure (second raw).

413 kPa	Time (s)	Force (N)	Depth (mm)	Width (mm)	Pressure (kPa)
Fig 4 E	0	0.113	0	0.4	**896.8**
a	0.0001	0.113	6.16	1.72	**48.6**
b	0.185	0.115	25.62	5.2	22. 2
c	0.37	0.123	31.22	6.5	18.9
d	0.555	0.128	35.99	10.06	12.7
e	0.74	0.134	40.04	11.89	11.3
620 kPa	Time (s)	Force (N)	Depth (mm)	Width (mm)	Pressure (kPa)
Fig 4 F	0	0.151	0	0.4	**1198**
a	0.0001	0.151	11.18	1.85	**56.3**
b	0.141	0.162	29.40	5.33	30.4
c	0.282	0.161	35.39	4.31	37.4
d	0.423	0.166	60.10	4.46	37.2
e	0.564	0.182	67.15	4.43	41.1

Supplementary Table 2 The model fitting based on previous study (*64*)

Pressure (psi)	Volume (mL)	m	b	Estimated depth(mm)	Actual mean depth(mm)	R^2
60	1	-0.0003	0.0947	84.6	48.3	7.84%
90	1	-0.0002	0.0926	129.2	51.8	13.69%
120	1	-0.0004	0.1753	38.9	72.6	20.53%
						RSMD = 54.2 mm

The discharge coefficient is calculated as follows:

We first collected the piston resisting force and displacement/time history at different piston injection rates (Fig S5). This data allowed us to calculate the actual and theoretical flow rate.

The actual jet volume flow rate could be calculated from piston injection rate and piston cross-sectional area.

$$Q = v_p * A_p \qquad \text{Eq (s1)}$$

Where v_p is the dynamic velocity during injection, A_p is the area of piston (d= 7 mm).

The theoretical maximum flow rate is calculated as follows:

$$P = F/A_p \qquad \text{Eq (s2)}$$

$$Q' = A_n\sqrt{\frac{2(P-P_0)}{\rho}} \qquad \text{Eq (s3)}$$

where F is the dynamic force of the piston during the injection. The relationship between the stable piston resistance force and the piston injection rate is shown in Fig S4 F. A_p is the area of the piston, A_n is the area of the nozzle orifice, P_0 is atmosphere pressure, ρ is the density of water.

The discharge coefficient is the actual flow rate divided by the theoretical flow rate.

$$Cd = \frac{Q}{Q'}$$

Eq (s4)

In addition, the Reynolds number of fluids was calculated based on the calculated Bernoulli velocity of the fluid.

$$R_e = D_p\mu_w\rho\sqrt{\frac{2(P-P_0)}{\rho}} \qquad \text{Eq (s5)}$$

Where D_p is the diameter of nozzle orifice, μ_w is the viscosity of water, ρ is the density of water, P_0 is atmosphere pressure, P is obtained from Eq (s2).

The relationships between Reynolds number and the discharge coefficient at different piston injection rates were presented in Fig S4 A-E.

Chapter 7 General conclusions

The discoveries of this thesis contributed towards understanding how high-speed liquid jets behave in the dental alveolar region and the risk factors for NFLJI to cause tissue damage. Our *in vitro* experiments explained the mechanism of tissue damage caused by NFLJI and proposed optimal techniques to minimize these complications. Meanwhile, our cadaveric studies and four pilot RCTs demonstrated that it is feasible to conduct a formal randomized controlled trial for comparing the clinical efficacy between NFLJIs and needle injections.

NFLJIs could be affected by various factors: the properties of injected fluid or the soft tissue and the NFLJI parameters, such as the injection angle, jet volume, and supply pressure. Among these factors, only the NFLJI parameters could be adjusted. To achieve predictable and successful NFLJI anesthesia in the oral cavity, it is essential to optimize the injection technique according to the unique characteristics of this technology and the anatomy of the injection sites.

Regarding the use of NFLJI for dental infiltration anesthesia, this thesis demonstrated that the unique anatomical structure of the dental alveolar region poses a unique challenge for liquid jet injections: The rigid bones of the jaws overlaying the thin oral mucosa could cause the high-speed liquid jet to rebound and regurgitate away from the injection site and lacerate the tissues. This thesis demonstrated that the problems could be in part mitigated by changing the injection angle. By modifying the injection angle from perpendicular to oblique angle, the vertical momentum of liquid jet reduced so that less stream impinges on bone surface and rebound, while the horizontal momentum of liquid jet increased, leading to more fluid disperse horizontally.

In terms of the dental nerve blocks, this thesis showed that the NFLJI can provide sufficient penetration depth to reach the main nerve branches innervating the oral cavity. It was

observed that the injection depth into oral soft tissue is directly proportional to the supply pressure and injection volume. However, higher NFLJI pressure could lead to higher penetration force on the tissue and increase the risk of tissue or nerve damage. This problem could be minimized by reducing the supply pressure.

In this work, we also presented a novel systematic approach for developing and translating research on NFLJI techniques from the laboratory to the cadaveric lab and ultimately to the clinical practice. The experimental setup with a high-speed camera, force transducer, motor stepper, and syringe pump allowed us to compare the injection outcomes of NFLJIs with needle injections *in vitro*. Using this approach, the mechanism of NFLJI and needle can be carefully investigated by controlling all the variables, such as properties of the soft tissue, the anatomical structure of the injection site, density and volume of the injected fluid, injection angle, pressure of NFLJI, needle insertion speed, and flow rate for needle injection. The cadaveric studies using multiple bodies validated the optimal technique developed in the laboratory. The pilot RCTs using a cross-over and split-mouth design confirmed the feasibility and safety of the optimized NFLJI techniques for infiltration and nerve block anesthesia. This thesis confirmed the feasibility for conducting a non-inferiority randomized control trial with a cross-over design to test whether optimized NFLJIs can achieve comparable clinical results to those obtained with to those of needle injections for dental infiltration or nerve blocks anesthesia.

Chapter 8 Limitations and future directions

The major limitation of the in vitro studies using ballistic gelatin is that its mechanical properties do not fully represent actual soft tissue. Ballistic gelatin was chosen to test NFLJIs because its density resembles that of human soft tissues. However, gelatin's fracture toughness and porosity are different from those of human soft tissue, which results in different reactions when the phantoms receive the NFLJI. For example, when disperses in gelatin, the jet flow with a vortex head creates a crack by shear force since gelatin is a non-porous material. In contrast, when dispersing in actual soft tissue, the jet diffuses through the porous structure instead of creating a crack. The difference in porosity results in smaller wound size and lower regurgitation volume in actual tissue than gelatine. Therefore, a novel oral soft tissue phantom material with comparable properties would be desirable. Porous materials such as chitosan or PHEMA with or without gelatin may provide proper porous structure and fracture toughness.

In our four pilot RCTs, the limited sample size cannot allow statistical assessment of differences between injection techniques. Therefore, the differences we observed clinically between perpendicular and oblique NFLJI or the differences between high-pressure and low-pressure NFLJI should be interpreted cautiously. A future RCT with a larger sample size would be needed to assess the efficacy and safety of the optimized NFLJI techniques for infiltration anesthesia or nerve blocks with enough statistical power.

Future RCTs should use a non-inferiority cross-over slit mouth design to minimize the required sample size and possible bias. Proper sample size could be 160 to 492 based on statistical simulation(α= 0.05, β= 0.2) (*174*) for assessment of efficacy of a new intervention compared to standard intervention in a non-inferiority cross-over study design. The primary outcomes are the efficacy and safety of NFLJI. Future studies using this design should report

efficacy, odds ratios of efficacy, and frequencies of concordant-discordant results per group. Mixed-model logistic regression should be deployed to analyze these data. The secondary outcomes are onset and duration of anesthesia and patients' experience in pain, anxiety, and taste. Normality tests and t-test should be deployed to analyze these data. Future studies should not use any topical anesthesia before injection. Future studies should employ oblique NFLJI for infiltration anesthesia and use a relatively low-pressure setup for nerve blocks to obtain the optimal anesthesia outcome with minimal complications.

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Chapter 10 Additional Publications

10.1 Al-Waeli, H., B. Nicolau, L. Stone, L. Abu Nada, **Q. Gao**, M. N. Abdallah, E. Abdulkader et al. "Chronotherapy of non-steroidal anti-inflammatory drugs may enhance postoperative recovery." Scientific reports 10, no. 1 (2020): 1-14.. https://doi.org/10.1038/s41598-019-57215-y

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Chronotherapy of Non-Steroidal Anti-Inflammatory Drugs May Enhance Postoperative Recovery

H. Al-Waeli[1], B. Nicolau[1], L. Stone[2], L. Abu Nada[2], Q. Gao[2], MN. Abdallah[3], E. Abdulkader[1], M. Suzuki[2], A. Mansour[2], A. Al Subaie[2] & F. Tamimi[2*]

Postoperative pain relief is crucial for full recovery. With the ongoing opioid epidemic and the insufficient effect of acetaminophen on severe pain; non-steroidal anti-inflammatory drugs (NSAIDs) are heavily used to alleviate this pain. However, NSAIDs are known to inhibit postoperative healing of connective tissues by inhibiting prostaglandin signaling. Pain intensity, inflammatory mediators associated with wound healing and the pharmacological action of NSAIDs vary throughout the day due to the circadian rhythm regulated by the clock genes. According to this rhythm, most of wound healing mediators and connective tissue formation occurs during the resting phase, while pain, inflammation and tissue resorption occur during the active period of the day. Here we show, in a murine tibia fracture surgical model, that NSAIDs are most effective in managing postoperative pain, healing and recovery when drug administration is limited to the active phase of the circadian rhythm. Limiting NSAID treatment to the active phase of the circadian rhythm resulted in overexpression of circadian clock genes, such as Period 2 (Per2) at the healing callus, and increased serum levels of anti-inflammatory cytokines interleukin-13 (IL-13), interleukin-4 (IL-4) and vascular endothelial growth factor. By contrast, NSAID administration during the resting phase resulted in severe bone healing impairment.

Postoperative recovery following invasive surgical interventions is usually painful and can cause significant morbidity and even mortality in some cases[1]. Postoperative pain is a response to injury in which inflammatory mediators are released, inflammatory cells infiltrate the damage site, and nociceptive nerve fibres are activated to produce pain[2]. Pain relief is crucial for full recovery; it is essential for the healing process and to resume physical activities[3]. However, drugs typically used for postoperative pain management are problematic. Acetaminophen is ineffective in severe pain, and while NSAID and opioids are useful for controlling surgical pain[4], opioids can cause constipation and addiction, and NSAID can delay healing[5–8]. Therefore, there is an urgent need for better strategies to manage postoperative pain. One way of achieving this is by developing NSAID treatments that control pain and inhibit inflammatory catabolic activities while sparing the anabolic pathways of wound healing.

Inflammation is essential in healing[9]. For example, when connective tissues are injured, an inflammatory response starts by the conversion of arachidonic acid (AA), either into prostaglandin H2 (PGH2) via cyclooxygenase (COX), or into interleukotrien A4 (LTA4) via 5-lipoxygenase (5-LO). Downstream specific synthetase enzymes convert PGH2 and LTA4 into bioactive lipid mediators, such as prostaglandin E2 (PGE2), which play a crucial role in tissue repair[9,10]. Also, release of pro-inflammatory cytokines and growth factors at the injury site, results in increased secretion of prostaglandins. Prostaglandins contribute to the regulation of mesenchymal cells[11], thus by inhibiting the production of PGE2 via COX, NSAIDs reduce the production of PGE2, and subsequently inhibit the healing of conective tissues (bone, cartillage, dermis, etc) (Table S1).

All living organisms possess a circadian rhythm that anticipates the response to changes during the 24-hour cycle[12]. The circadian system in mammals is composed of a central clock within the suprachiasmatic nuclei and peripheral clocks inside all cells. The circadian clock is controlled through a feedback loop of heterodimer core clock genes composed of circadian locomotor output cycles kaput(Clock) and brain and muscle Arnt such as protein-1(Bmal1). Clock and Bmal1 drive the expression of two inhibitors, cryptochrome(Cry) and period(Per)[13]. This molecular clock modulates the immune response and the healing processes in connective tissues[13] (Fig. 1

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10.2 Al-Hamed, F. S., A. Hijazi, **Q. Gao**, Z. Badran, and F. Tamimi. "Platelet Concentrate Treatments for Temporomandibular Disorders: A Systematic Review and Meta-analysis." JDR Clinical & Translational Research 6, no. 2 (2021): 174-183.https://doi.org/10.1177%2F2380084420927326

JDR Clinical & Translational Research April 2021

REVIEWS

Platelet Concentrate Treatments for Temporomandibular Disorders: A Systematic Review and Meta-analysis

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Abstract: ***Objectives:*** *This systematic review compared platelet concentrates (PCs) versus hyaluronic acid (HA) or saline/Ringer's solution injections as treatments of temporomandibular osteoarthritis and disc displacement in terms of pain and maximum mouth opening (MMO).*

Methods: *PubMed, Cochrane, and Scopus were searched up to March 6, 2020. Inclusion criteria were randomized clinical trials (RCTs). Exclusion criteria were case series, observational studies, animal studies, and reviews. The Effective Public Health Practice Project (EPHPP) quality assessment tool was used to assess the risk of bias in the included studies. The weighted mean difference was used to compare the results.*

Results: *Nine RCTs were included with a total of 407 patients. The numbers of joints treated were 262, 112, and 112 in the PC, HA, and saline groups, respectively. The quality of studies was rated as strong in 4 studies, moderate in 4 studies, and weak in 1 study. The meta-analysis revealed that PCs decreased pain visual analogue scale (VAS) scores compared to HA by an average of −1.11 (CI, −1.62 to −0.60; P < 0.0001) and −0.57 (CI, −1.55 to 0.41; P = 0.26) at 3 and 12 mo follow-up respectively. Also, the average decrease in pain scores with PC compared to saline was −1.33 (CI, −2.61 to −0.06; P = 0.04), −2.07 (CI, −3.46 to −0.69; P = 0.003), and −2.71 (CI, −4.69 to −0.72; P = 0.008) at 3, 6, and 12 mo, respectively. Regarding MMO measurements, PC was comparable to HA, but it was significantly better than saline after 3 and 6 mo [2.9 mm (CI, 1.47 to 4.3; P < 0.0001), and 1.69 mm (CI, 0.13 to 3.25; P = 0.03) respectively].*

Conclusion: *PC reduces pain VAS scores compared to HA during the first 3 m after treatment, and when compared to saline, it reduces pain and increases MMO for longer durations. However, due to differences between groups regarding PC preparation protocols and study heterogeneity, further standardized RCTs are required.*

Knowledge Transfer Statement: *This study provides researchers and clinicians with quantitative and qualitative analyses of the current evidence regarding the clinical outcomes of platelet concentrate injections in the treatment of temporomandibular joint osteoarthritis and disc displacement in terms of pain control and maximum mouth opening.*

Keywords: joints, mastication, osteoarthritis, pain, platelet-rich plasma, temporomandibular joint

Introduction

Temporomandibular disorders (TMDs) are diseases of multifactorial origin that affect temporomandibular joint (TMJ) articular surfaces as well as the surrounding masticatory muscles (Ahmad and Schiffman 2016). Myofascial pain dysfunction syndrome, disc displacement, joint osteoarthritis, hypermobility, dislocation, and ankylosis are among the most common TMDs. Disc displacement is an abnormal position of the articular disc in relation

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A supplemental appendix to this article is available online.

10.3 Kolosova, Ksenia, **Qiman Gao**, Marius Tuznik, Sarah Bouhabel, Karen M. Kost, Huijie Wang, Nicole YK Li-Jessen, Luc Mongeau, and Paul W. Wiseman. "Characterizing Vocal Fold Injury Recovery in a Rabbit Model With Three-Dimensional Virtual Histology." The Laryngoscope (2020). https://doi.org/10.1002/lary.29028

Characterizing Vocal Fold Injury Recovery in a Rabbit Model With Three-Dimensional Virtual Histology

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Objectives/Hypothesis: In animal studies of vocal fold scarring and treatment, imaging-based evaluation is most often conducted by tissue slicing and histological staining. Given variation in anatomy, injury type, severity, and sacrifice timepoints, planar histological sections provide limited spatiotemporal details of tissue repair. Three-dimensional (3D) virtual histology may provide additional contextual spatial information, enhancing objective interpretation. The study's aim was to evaluate the suitability of magnetic resonance imaging (MRI), microscale computed tomography (CT), and nonlinear laser-scanning microscopy (NM) as virtual histology approaches for rabbit studies of vocal fold scarring.

Methods: A unilateral injury was created using microcup forceps in the left vocal fold of three New Zealand White rabbits. Animals were sacrificed at 3, 10, and 39 days postinjury. ex vivo imaging of excised larynges was performed with MRI, CT, and NM modalities.

Results: The MRI modality allowed visualization of injury location and morphological internal features with 100-µm spatial resolution. The CT modality provided a view of the injury defect surface with 12-µm spatial resolution. The NM modality with optical clearing resolved second-harmonic generation signal of collagen fibers and two-photon autofluorescence in vocal fold lamina propria, muscle, and surrounding cartilage structures at submicrometer spatial scales.

Conclusions: Features of vocal fold injury and wound healing were observed with MRI, CT, and NM. The MRI and CT modalities provided contextual spatial information and dissection guidance, whereas NM resolved extracellular matrix structure. The results serve as a proof of concept to motivate incorporation of 3D virtual histology techniques in future vocal fold injury animal studies.

Key Words: Vocal fold scarring, multimodal imaging, magnetic resonance imaging, computed tomography, nonlinear microscopy.

Level of Evidence: NA

***Laryngoscope*, 131:1578–1587, 2021**

INTRODUCTION

Vocal fold scars may form following a disruption of vocal fold tissue structure, including surgical excision of benign and malignant lesions.[1,2] A large inflammatory response to such a traumatic event may lead to an incomplete wound healing response with insufficient extracellular matrix (ECM) remodeling.[3] When a scar is formed, extensive cross-linking between ECM components, in addition to greater amounts of disorganized collagen, increases tissue stiffness and hampers vocal fold vibration for phonatory functions.[4] Treatment of vocal fold injury and management of vocal fold scars remain active areas of research. At present, various prophylactic treatments for iatrogenic vocal fold scarring have been proposed.[5] Treatment approaches undergoing preclinical investigation include injectable biomaterials such as collagen,[6] hyaluronic acid,[7,8] and other hydrogels such as chitosan,[9] tissue engineering,[10] and stem cell therapy.[11]

Small animal models including rats,[12] ferrets,[13] and rabbits[14] are used to evaluate immune response to the treatment and as an initial indicator of efficacy in treatment or prevention of vocal fold scarring. Rabbits are frequently chosen because their vocal folds are larger than those of rats, which facilitates phonomicrosurgery and biomaterial injection/implantation. Rabbit vocal fold scarring

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Editor's Note: This Manuscript was accepted for publication on July 28, 2020.

This work was scheduled to be presented orally at the Annual Meeting of the American Laryngological Association at the Combined Otolaryngology Spring Meetings, Atlanta, Georgia, U.S.A., April 22–26, 2020 (meeting canceled).

This work was supported by the National Institute for Deafness and Other Communication Disorders of the National Institutes of Health under award number R01DC005788 and Natural Sciences and Engineering Research Council under award number RGPIN-2018-03843.

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

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DOI: 10.1002/lary.29028

10.4 Ma, Zhenwei, Zhen Yang, **Qiman Gao**, Guangyu Bao, Amin Valiei, Fan Yang, Ran Huo et al. "Bioinspired tough gel sheath for robust and versatile surface functionalization." Science Advances 7, no. 15 (2021): eabc3012.DOI: 10.1126/sciadv.abc3012

SCIENCE ADVANCES | RESEARCH ARTICLE

APPLIED SCIENCES AND ENGINEERING

Bioinspired tough gel sheath for robust and versatile surface functionalization

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Sutures pervade surgeries, but their performance is limited by the mechanical mismatch with tissues and the lack of advanced functionality. Existing modification strategies result in either deterioration of suture's bulk properties or a weak coating susceptible to rupture or delamination. Inspired by tendon endotenon sheath, we report a versatile strategy to functionalize fiber-based devices such as sutures. This strategy seamlessly unites surgical sutures, tough gel sheath, and various functional materials. Robust modification is demonstrated with strong interfacial adhesion (>2000 J m^{-2}). The surface stiffness, friction, and drag of the suture when interfacing with tissues can be markedly reduced, without compromising the tensile strength. Versatile functionalization of the suture for infection prevention, wound monitoring, drug delivery, and near-infrared imaging is then presented. This platform technology is applicable to other fiber-based devices and foreseen to affect broad technological areas ranging from wound management to smart textiles.

INTRODUCTION

Sutures are a class of fiber-based devices primarily to mechanically approximate tissues or attach wearable/implantable devices to human body (*1*). They are in the form of either mono- or multifilaments (i.e., braided) and designed to degrade or stay permanently in the body. A variety of materials have been invented and adopted as surgical sutures, including plastics (degradable: polyglycolide and polylactic acid; nondegradable: nylon and polypropylene), biologically derived proteins (collagen and silk), and metals (stainless steel and nitinol). The sutures have been widely used in many branches of medicine such as wound closure and anastomosis with a global market of over 5 billion U.S. dollars (*2*). Despite the recent progress of tissue adhesives (*3*–*7*), they will remain indispensable for general surgical procedures because of their reliable performance, ease of implementation, and the capacity to exert larger forces than any tissue adhesives (*2*).

However, the performance of existing sutures has been limited by their poor biomechanical properties and lack of functionality, which are implicated in surgical and postsurgical complications. First, sutures are made of rigid dry materials (elastic modulus of >1 GPa) in contrast to soft hydrated tissues (elastic modulus of <100 kPa), as they need to carry substantial mechanical loading along the axis to approximate tissues (*8*). This mechanical mismatch is found to cause inflammation and impaired healing outcomes (*9*). Second, the rough surface of sutures, particularly for the braided sutures, can drag and rub against the contacting tissue during and after suture placement. This mechanical irritation can damage fragile tissues and those under disease conditions such as aneurysm and ulcer, leading to tissue dissection and other postsurgery complications (*10*, *11*). In addition, clinically used sutures lack advanced functionality for wound management. Thus, multifunctional sutures are in demand to perceive, report, and respond to the wound healing process, for instance, delivering therapeutic to promote wound healing (*12*) and preventing surgical site infections (*13*). These functional sutures are developed recently, which feature drug delivery or sensing capacities. There are also drug-eluting or antibacterial sutures commercially available (e.g., Coated VICRYL Plus Antibacterial, Ethicon), capable of releasing drug or antibacterial compounds (*14*). However, limitations to these approaches remain, including complex fabrication process, high cost, limited physical integrity, and the abovementioned biomechanical constraints. These issues associated with surgical sutures are also found in other fiber-based devices, particularly those interfacing with the human body such as guidewires and smart textiles. New strategies to improve the biomechanical properties, functionality of sutures, and other fiber-based devices continue to be sought.

General strategies to functionalize sutures include bulk modification and surface functionalization. The former involves bottom-up approaches to (re)produce the suture (e.g., electrospinning and melt extrusion), which may compromise the suture's strength and are inapplicable to commercially available sutures (*15*, *16*). To minimize the alteration of the bulk properties, the surface functionalization is appealing, which results in a suture coating via dip coating/soaking (*17*, *18*), layer-by-layer deposition (*19*, *20*), grafting (*21*–*23*), and impregnation (*24*, *25*). However, the suture coating is often weak and vulnerable to fragmentation and delamination, due to the chemical inertness of suture materials and the demanding mechanical loading of the suture application (e.g., shear and compression during suturing and knotting). The mechanical failure of suture coating results in the loss of functionality (fig. S1, A to C) and other side effects (e.g., burst drug release for drug-eluting suture coating). Evidently, the toughness and adhesion of the suture coating is thus mission critical and recognized as a prerequisite of any reliable functionalization.

For surface functionalization of surgical sutures, hydrogel technologies are promising in light of recent developments of tough

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10.5 Al-Hamed, Faez Saleh, Ola M. Maria, Jeff Phan, Ahmed Al Subaie, **Qiman Gao**, Alaa Mansour, Lina Abu Nada et al. "Postoperative administration of the acetylcholinesterase inhibitor, donepezil, interferes with bone healing and implant osseointegration in a rat model." Biomolecules 10, no. 9 (2020): 1318. https://doi.org/10.3390/biom10091318

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Article

Postoperative Administration of the Acetylcholinesterase Inhibitor, Donepezil, Interferes with Bone Healing and Implant Osseointegration in a Rat Model

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Received: 3 August 2020; Accepted: 10 September 2020; Published: 14 September 2020

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Abstract: Donepezil is an acetylcholinesterase inhibitor commonly used to treat mild to moderate Alzheimer's disease. Its use has been associated with increased bone mass in humans and animals. However, the effect of postoperative administration of donepezil on bone healing remains unknown. Therefore, this study aimed to assess the impact of postoperative injection of donepezil on bone healing, titanium-implant osseointegration, and soft tissue healing. Twenty-two Sprague-Dawley rats were randomly assigned to receive a daily dose of either donepezil (0.6 mg/kg) or saline as a control. In each rat, a uni-cortical defect was created in the right tibia metaphysis and a custom-made titanium implant was placed in the left tibiae. After two weeks, rats were euthanized, and their bones were analysed by Micro-CT and histology. The healing of bone defect and implant osseointegration in the rats treated with donepezil were significantly reduced compared to the saline-treated rats. Histomorphometric analysis showed lower immune cell infiltration in bone defects treated with donepezil compared to the saline-treated defects. On the other hand, the healing time of soft tissue wounds was significantly shorter in donepezil-treated rats compared to the controls. In conclusion, short-term administration of donepezil hinders bone healing whereas enhancing soft tissue healing.

Keywords: acetylcholinesterase inhibitors; bone healing; osseointegration; donepezil; hemostasis

1. Introduction

Bone remodeling is a continuous process of bone resorption by osteoclasts followed by bone formation by osteoblasts [1]. It is regulated locally through direct interactions between osteoclasts,

10.6 Al-Hamed, F.S., Abu-Nada, L., Rodan, R., Sarrigiannidis, S., Ramirez-Garcialuna, J.L., Moussa, H., Elkashty, O., **Gao, Q.,** Basiri, T., Baca, L. and Torres, J., 2021. Differences in platelet-rich plasma composition influence bone healing. Journal of Clinical Periodontology. DOI: 10.1111/jcpe.13546

ORIGINAL ARTICLE

Journal of Clinical Periodontology WILEY

Differences in platelet-rich plasma composition influence bone healing

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Funding information
Faez Saleh Al-Hamed was supported by a scholarship from Al Awn Foundation for Development, Yemen; Ph.D. training award from Funds de Recherche Québec—Santé (FRQS: 257709); and the Alpha Omega Foundation of Canada grant (2018 and 2019). He also received the Graduate Excellence Award from the Faculty of Dentistry, McGill University. Lina Abu-Nada was supported by Ph.D. training award from Funds de Recherche Québec—Santé and RSBO. Marie Lordkipanidzé is a Canada Research Chair in Platelets as Vectors and Biomarkers. The authors also acknowledge support from the Canada Research Chair Program and Le Réseau de recherche en santé buccodentaire et osseuse (RSBO).

Abstract

Aim: Platelet-rich plasma (PRP) is an autologous blood-derived material that has been used to enhance bone regeneration. Clinical studies, however, reported inconsistent outcomes. This study aimed to assess the effect of changes in leucocyte and PRP (L-PRP) composition on bone defect healing.

Materials and Methods: L-PRPs were prepared using different centrifugation methods and their regenerative potential was assessed in an in-vivo rat model. Bilateral critical-size tibial bone defects were created and filled with single-spin L-PRP, double-spin L-PRP, or filtered L-PRP. Empty defects and defects treated with collagen scaffolds served as controls. Rats were euthanized after 2 weeks, and their tibias were collected and analysed using micro-CT and histology.

Results: Double-spin L-PRP contained higher concentrations of platelets than single-spin L-PRP and filtered L-PRP. Filtration of single-spin L-PRP resulted in lower concentrations of minerals and metabolites. In vivo, double-spin L-PRP improved bone

Chapter 11 Supplementary documents:
Research proposal, IRB approval, and questionnaires for clinical trials

RESEARCH PROTOCOL	The Dental Application of Needle-free Device: A Pilot Study
Title	
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A. ABSTRACT

Many patients experience pain and anxiety from traditional dental anesthesia with needles and thus may avoid necessary dental treatments. These problems could be solved by the needle-free device. It delivers drug solutions by creating a micro-thin pressure liquid jet to penetrate the skin and disperse in the soft tissue. The needle-free device can provide better local anesthesia treatment with many advantages like eliminating injection pain, needle phobia and needle disposal, reducing drug volume and increasing drug diffusion according to preliminary laboratory study, which would benefit both the patient and the clinician. However, anesthesia techniques in dentistry were all developed for needle injection, and they are not very effective with the needle-free device. We aim to understand and optimize the dental anesthesia technique with the needle-free device.

We have done a series of laboratory experiment to develop our new technique for the needle-free device. The laboratory experiments showed that needle-free device provides sufficient penetration distance for dental anesthesia. But there is lacking clinical data about the effectiveness. In this study, we aim to compare the efficacy of needle-free anesthesia with needle anesthesia and validate our technique on cadavers as well as on voluntary participants. Ultimately, we aim to provide a clinical guideline for the dentist.

This study has two stages. Stage I will be a series of cadaver study executed at the anatomy lab in Strathcona building, aims to compare the efficacy of needle-free and needle anesthesia and validate our technique. Stage II will be a clinical study executed in the Dental clinic of McGill University Faculty of dentistry. Participants will be assigned to one of the following four groups: 1. Infiltration on a maxillary lateral incisor,2 Nerve block: 2.1 Inferior alveolar nerve block, 2.2 Mental nerve block 2.3. Infraorbital nerve block. In each group, there are two sides to receive injections for comparison. For the first group, the aim is to compare two different infiltration techniques for the needle-free device: Vertical to bone injection Vs. Parallel to bone injection. For the latter group, the aim is to compare the effectiveness of needle-free anesthesia with conventional needle anesthesia. To minimize the influence between two injections, we will wait for 30 to 40 minutes until the anesthesia effect end, and the participants forget the experience. The needle-free injection will be performed by an experienced dentist according to our protocol. Needle injection will be performed by an experienced dentist according to standard clinical injection technique. After injection, the duration of anesthesia, patient feeling of taste and pain

will be recorded during anesthesia as the outcome. Participants will stay in the clinic until the anesthesia effect disappear for 20 min and feel comfortable to leave. Any complications during and after the anesthesia or will be recorded. After one week, we will contact all participants by phone call or email to ask about any possible complications.

B. Research Outline

Background: Many patients experience pain and anxiety from dental injections and as a result, may avoid necessary dental treatments [1-3]. This dental phobia and stress from needle injections are common worldwide [1, 2], especially in children[3]. Research shows that children exposed to treatment involving local anesthesia have high odds of displaying negative behavior[4]. Administering the dental anesthesia without using needle would reduce this phobia and manage the pain and anxiety associated. Most patients do not differentiate between the feeling of anxiety and the true pain experience [5]. The pain associated with injections is caused by irritation of mucosa from the anesthetic formulation, sensitivity of the injection site, mechanical trauma caused by piercing the tissue, and distension resulting from injecting the contents of the syringe [6]. Improper technique is considered to be one of the major factors contributing to the failure of the desired result. However, the conventional technique is reported to have been associated with risks and complications such as neural or vascular injury, intravascular injection, and failure to achieve adequate anesthesia [7].

Nowadays, needle-free devices are wildly used in dermatology [8]and vaccination[9] fields with many advantages like eliminating injection pain, needle phobia and needle disposal, preventing needle trans-infection, reducing drug volume and increasing drug diffusion, which would benefit both the patient and the clinician. The applications in dermatology and vaccination clinical trials proved its safety and success.

This alternative needle-free, pain-free anesthesia would benefit both the patient and the clinician and would be a remarkable achievement in the dentistry field. From this prospect, we are working closely with Medical International Technologies Company to use their new needle-free device—Meso Jet (MIT CANADA, MIT-D3-03), which is a new needle-free device to deliver the local anesthetic. The Meso-Jet is a precision medical device, designed for the medical field to deliver injections of drug solutions without a needle. By creating a micro-thin pressure jet, the liquid penetrates through the skin and disperses in the soft tissue. This device is approved by HEALTH CANADA and the International Organization for Standardization.

Within so many advantages, the needle-free devices are still limited to be applied in dental anesthesia due to controversy. Previous clinical trials stated that though the injection pain of needle-free device is significantly less than conventional needle injection [10, 11], the efficacy of needle-free anesthesia remains unclear and poorly investigated. Some studies demonstrated that needle-free anesthesia is a good alternative for conventional needle anesthesia [12] or more acceptable among adults [11]. However, some studies claimed the needle-free device is less accepted or preferred by 6-11 year-olds children [13], mainly due to bad taste caused by drug rebound, noise generated by needle-free device and insufficient pulp anesthesia. This controversy has also been found from the needle-free anesthesia in the non-dentistry field [10, 14, 15]. All the above-mentioned needle-free anesthesia clinical trial publications haven't described how they perform the anesthesia.

The mechanism of needle-free device is different from mechanism of needle injection; therefore, the injection direction, site, angel need to be modified based on the mechanism of the device when performing injection. Currently, there is no study investigated both the mechanism and optimal technique for needle-free anesthesia. We have done a series of laboratory experiments to understand the needle-free device and develop our optimal technique for the needle-free anesthesia [16]. The laboratory experiments showed that needle-free device provides sufficient penetration distance for dental anesthesia. A cadaver study and a clinical pilot study are needed to assess and compare the effect of needle-free anesthesia and conventional needle anesthesia.

Objectives:

Stage I:

To compare the efficacy and disperse effect of needle-free anesthesia with needle anesthesia on cadavers by a randomized split-mouth study.

To understand the anatomical variance of injection site by CT analysis.

Stage II:

1. Primary objective: To test the effectiveness of our needle-free anesthesia with new injection technique.

2. Secondary objectives:

a. Infiltration: Compare and optimize the needle-free anesthesia technique that we have developed.

(The next step, we want to compare the efficacy of needle-free anesthesia with needle anesthesia)

b. Nerve block: evaluate the effectiveness by comparing the needle-free anesthesia with needle anesthesia.

c. To develop a user guide for the dentists.

C. Clinical importance:

Local dental anesthesia is required in many dental procedures. The needle-free device will benefit both the patient and the clinician by eliminating injection pain, needle phobia, and needle disposal, preventing needle trans-infection, reducing drug volume and increasing drug diffusion. There are 300 million local anesthetic cartilages are consumed worldwide every year [17]and cost a big amount of money for the government to deal with medical sharps. Development of needle-free devices will help in the reduction of stress and anxiety caused by needle injection as a first step of the treatment. Also, this device will help in preventing the infection and injuries which are usually major complications from needled dental anesthesia. Furthermore, such a device could be used to deliver other therapeutic materials to the targeted tissue with a conservative approach. The world market of dental local anesthetics is as high as 4 billion dollars, this project also helps the Canadian economy by reducing the cost of the disposable needle and the operation time, helps eliminate the need of sedation and straps, avoids the risk of childhood death caused by dental sedation [18]. The ultimate benefit of this study is to promote the patients' access to dental care.

D. RESEARCH PLAN AND APPROACH

1 Stage I:

1.1 Comparison the efficacy and disperse effect of needle-free anesthesia and needle anesthesia on cadaver.

1.1.1 Experiment design

To compare the efficacy, success rate, and disperse effect of needle-free anesthesia with needle anesthesia, we plan to conduct a randomized split-mouth study on cadavers. Each cadaver has two sides for the same nerve block site. Each cadaver will receive nerve blocks with 0.3ml 10% methylene blue by either needle (group 1) or needle-free (group 2) randomly at two side in the mouth at the following nerve block sites: infraorbital nerve, mental nerve, great palatine,

nasopalatine nerve, inferior alveolar nerve, lingual nerve and buccal nerve. These nerve block sites are selected because these nerves are visible, able to be dissected and recorded.

1.1.2 Materials

Cadavers are free donated to McGill University department of anatomy and cell biology for student training. The cadavers are preserved in the Thiel preservation, which has been found to demonstrate efficacy in tissue quality, elasticity, and handling in addition to playing a role in teaching and training[19]. After the training workshop, the cadavers will be used for our study. Methylene blue will be used to mimic the local anesthesia, and the blue color will make the disperse area visible, which is a commonly used method in the cadaver anesthesia technique study [20].

MESO-JET needle-free injection system is offered by the company for free. The operator received training from the company before the experiment.

A digital camera is used to record the result after injection and dissection. A ruler is used when taking the picture to help measure the injection effect.

1.1.3 Method

Cadavers will first be randomized on the sequences and interventions on each nerve block site by Microsoft Excel. The operator performs injection according to randomization orders on each site (infraorbital nerve, mental nerve, great palatine, nasopalatine nerve, inferior alveolar nerve, lingual nerve, and buccal nerve). The operator received training from the company before the experiment. After each injection with either needle or needle-free anesthesia, the dissection will be performed by an expert in the department of anatomy. The injection method for both needle or needle-free will follow the description of anesthesia technique attached as supplemental materials.

All the test results were recorded by digital camera. Pictures will be taken with ruler, the identical part on the face will be covered to protect the cadaver’s privacy. 2-3 different assessors with oral surgery background will participant to confirm whether the nerve is blocked by the needle-free injection, by checking whether the dissected nerves are surrounded by methylene blue in the photos. The assessors are blinded on which intervention is used. After the experiment, all the equipment will be cleaned in soapy water.

1.1.4 Sample size and statistics

A minimum number of 6 cadavers is needed to provide the statistic power. We are looking forward to collecting more than 12 cadavers to provide stronger evidence. Considering the possible failure or accident with the device or dissection or cadaver, we will set the number at 16.

1.1.5 Inclusion and exclusion: Only Thiel's body will be used in this study. Body must have integral maxillae and mandible with the integral soft tissue on the face. Since the age of the donors is most likely to be old, cadaver with or without teeth will both be included first; if cadavers without any teeth have more difficulties in getting a reproducible result, we may exclude them.

1.1.6 Ethic Consideration.

Cadavers are free donated to McGill University department of anatomy and cell biology for student training. The experiment will be conducted after the training session. All the identical parts of the body will be covered when record the experimental results to protect the privacy of our donors.

1.2 Understand the anatomy variance of injection site by CT analysis.

1.2.1 Experiment design and methods

The inferior alveolar nerve block(IANB) is the most challenging local anesthesia technique in dental anesthesia[21].In our cadaver experiment, we have successfully archived IANB many times; however, in the dental clinic, we have faced many failure cases because there are anatomical variances among patients which limited the technique to be applied.

To investigate the anatomical variance of human mandible, a Cone Beam Computed Tomography (CBCT) analysis is designed. CBCT data are obtained from McGill Student and Staff Dental Clinic, Department of Oral & Maxillofacial Radiology. Dragonfly (Object Research Systems, Montreal, Canada) will be used to reconstruct the CBCY and analyze the data.

In Each CBCT data, the mandible will be reconstructed and analyzed according to the following craniofacial anatomy landmarks: a. the inferior alveolar foramen b. the highest point of lingula c. the lowest point of mandibular notch d. the occlusal plane for mandible e. the inner plane of ramus f. the external plane of ramus g. pterygomandibular space/plane h. Triangle-G (defined as the triangle formed by the lingual edge of mandible also called coronoid notch, the pterygomandibular raphe also called pterygomandibular ligament, and the medial pterygoid muscle, this triangle is the entrance of pterygomandibular space)

The following relationship will be measured: 1. the distance between (a) and (c), 2. the distance between (a) and (b) 3. the distance between (a) and (h), 4. the angle between (d) and (g), 5. the angle between (e) and (f).

The data analyzers are experienced dentists trained by our oral & maxillofacial radiology specialist Dr. Didem.

1.2.2 Sample size and statistics

We will need 150-200 CBCT data to effectively generate a human mandible morphology model. Previous literature [22, 23] with similar study objectives used a sample size of 100-200. The gender and age difference[22] will be considered and compared. The outcome will be a morphology model and measurement data of human mandible according to different gender and age group.

1.2.3 Ethic Consideration.

The patients who came to McGill Student and Staff Dental Clinic has already signed their consent form to provide their oral examination data for research purpose, and we will use these available CBCT data.

2 Stage II: Comparison of the efficacy of dental anesthesia with needle-free device and needle: A pilot clinical trial.

2. 1 Trial design, recruitment, and clinical procedure

We will conduct pilot split-mouth cross-over randomized clinical trial to compare the efficacy of injection technique for needle-free anesthesia in voluntary adult participants.

Recruitment and consent: We will post our recruitment ads in the dental clinic, on the Facebook group, and through faculty mail list. All the participant will be gathered to inform about the research study, its nature, and purpose and sign the consent form to join the trial. Patients will be given an opportunity to ask questions and to take the research consent form home for further consideration. Those interested in taking part in the study will be asked to sign the form. The trial will be executed in the Dental clinic of McGill University Faculty of dentistry.

Group design: After signing the consent form, the participants will be assigned to a random number, which decides the group and the sequence of the procedure. Selected subjects will be assigned to five groups with three different anesthesia technique:

1. Infiltration anesthesia: infiltration on maxillary lateral incisor. 1.1 1.2

2. Nerve block: 2.1. Inferior alveolar nerve block 2.2. Mental nerve block 2.3. Infraorbital nerve block

Each participant has two sides (left and right side) in their mouth to receive one type of anesthesia; we choose the side randomly to be the control and intervention side.

For the group 1.1, the aim is to compare two different infiltration techniques for the needle-free device: Perpendicular to bone injection Vs. Parallel to bone injection. The subjects will receive a needle-free injection with local anesthetic, use parallel to bone injection method on the maxillary lateral incisor at the intervention side. The injection method for both needle or needle-free will follow the description of anesthesia technique attached as supplemental materials.

To minimize the influence between two injections, we will wait for 30 minutes until the anesthesia effect end, and the participants forget the experience. Then the subjects will receive a needle-free injection local anesthetic, use perpendicular to bone injection method on the maxillary lateral incisor at control side.

For the group 1.2 and group 2, the aim is to compare the effectiveness of needle-free anesthesia with conventional needle anesthesia on nerve block. The subjects will receive a needle-free injection local anesthetic on the infiltration at upper lateral incisor (1.2), inferior alveolar nerve (group2.1) or mental nerve (group 2.2) or infraorbital nerve (group 2.3) block at intervention side. Then the subjects will receive a needle injection with local anesthetic, use the conventional nerve block methods[21] at the control side. The injection method for both needle or needle-free will follow the description of anesthesia technique attached as supplemental materials. To minimize the influence between two injections, we will wait for 30 minutes till the anesthesia effect end, and the participants forget the experience.

Selection of injection site: we select the four most commonly used technique here in our study design -infiltration, mental, infraorbital and inferior alveolar nerve block- to provide the strongest evidence in comparing the efficacy.

The needle-free injection will be performed by an experienced dentist according to our new developing technique. Needle injection will be performed by an experienced dentist according to standard clinical injection technique[21]. 2 % Lidocaine with 1:100,000 epinephrine local anesthetics will be used in both methods of injection. The needle-free device is provided by Medical International Technologies Company (MIT CANADA, MIT-D3-03). This device is approved by HEALTH CANADA and the International Organization for Standardization.

Outcome assessment: After injection, we will evaluate the effect of anesthesia by the following method:

a. The duration of anesthesia will be calculated and recorded by using a probe to pinch the soft tissue in the area of administration to evaluate the numbness.

b. The potency of anesthesia will be tested by electrical dental pulp test to check how many dental pulps is under anesthesia. Both the cold pulp test and electronic pulp test have been widely used to assess the effect of anesthesia in the dental clinic before treatment[21, 24]. We will use the electric pulp test, which is more accurate and objective comparing to cold pulp test [25]. Use of electrical pulp testing with no response from the tooth with maximal EPT output (80/80) indicating sufficient anesthesia before dental treatment[21].

c. Participants' pain level and anxiety level before and during injection will be recorded by filling out a questionnaire to evaluate the injection pain. Pain questionnaire refers to the numeric rating visual analysis scale in pain[26]. Anxiety visual analysis scale questionnaire with a 100-mm scale has been massively used in pain or anesthesia research and could effectively measure patients' anesthetic concerns[27]. We won't record the pain and anxiety level after injection since there is no dental treatment after injection and the participant won't feel any pain or anxiety after the injection.

d. Participants' feelings on the taste will be recorded by the 9-point hedonic scale. The 9-point hedonic scale has been used routinely in food science for 60 years[28].

e. Any sign of adverse effect related to needle anesthesia or needle-free anesthesia will be recorded as the negative effect.

Participants will stay in the clinic until the anesthesia effect disappear for 20 min and feel comfortable to leave. Any complications during and after the anesthesia or will be recorded. After one week, the follow-up coordinator will call or email each participant to ask if there any possible complication happen after injection and record the detail.

2.2 Sample size and Statistics

We set the sample size at 6 for each group in this pilot study to provide minimum statistical power to compare the efficacy of needle and needle-free anesthesia.

The preliminary data will be description analysis. Age, gender, education, anesthesia duration (minutes the anesthesia last), potency (number of dental pulps under anesthesia), pain level before and during injection, participants' feeling on the taste and other feedback will all be

recorded in the questionnaire with the help of assistants. This preliminary data will be used to define future sample size.

We will stop recruitment once we reach 30 participants or there is any severe complication happen, such as irreversible nerve or soft tissue injury (e.g., nerve paralysis for more than three weeks without any recover).

If the participant is found to have allergy history to any materials in this study, including metal or anesthetic drug, the researcher might terminate his or her participation in this study for the sake of participants' health.

2.3 Blinding and randomization: Patient will all be randomized, then receive interventions of needle-free or needle anesthesia on either side. There is a washout period of 30 min between the end of first injection and the start of second injection. We justify that 30 min is enough for subjects to forget the very mild pain feeling (pain score at 1-3 out of 10). The injection of needle-free anesthesia and needle anesthesia will be performed by a trained dentist to minimize the operator's difference. Followed by a questionnaire session and soft tissue probe test to record the initial anesthesia time and other tests according to the flow chart. Though it's impossible to blind the anesthesia operator and patients, the questionnaire assessors and the data analysts will be blinded.

Randomization will be carried out off-site by a statistician with Excel Randomized Number at the Department of Dentistry, McGill University. After screening and clinical examinations, all participants enrolled in the study will be randomly assigned to receive the intervention (Needle-free) or control (needle) injection in the first and vice versa in the second.

2.4 Inclusion Criteria

To be considered for inclusion in the study patients must:

1) Young adult, aged at 18~35 years-old,

2) Can read, write and speak English fluently to understand and fill out the consent form and questionnaire

3) Has an adequate understanding of their participation.

4) Cooperate, willing to sign the consent form

5) Should not have had any root canal therapy at canines, up lateral incisors and lower premolar and molars.

2.5 Exclusion Criteria

Subjects will be excluded from participating in the study if:

1) Age younger than 18 or older than 35
2) Can't read, write and speak English fluently,
3) Uncooperative, unwilling to participate in the study.
4) Has major systemic health problems or mental health problem, health conditions that may need special precautions

2.6 Confidentiality

Participants' ID will not be disclosed either in files nor photographs. Photographs will be taken only for the oral area with permission. The participant will be anonymous and assigned to a random number. Participants' name and email addresses are needed for the follow-up. All the filled questionnaires and records will be stored in a locked file cabinet; the electronic data will be kept in a password protected file on a computer. Only the researchers related to this study will have access. All the personal information will be kept apart from the data and stored in a secure place. This information will be kept for seven years after the study is terminated. After admission to the study, a subject may withdraw at any time for any reason.

2.6 Ethical considerations

The study will be conducted according to ethical principles stated in the Declaration of Helsinki (2013) [11], ethics approval will be obtained before initiating the study, consent forms will take into consideration the well-being, free-will, and respect of the participants, including respect of privacy.

E. Feasibility and pertinence

The team brought together in this project has all the expertise and access to instruments and facilities required for completing this project. Pertinence: All members of the team are accredited dentists with experience in clinical research.

Dr. Faleh Tamimi is a licensed dentist (by the ORD De Dentists Du Quebec) and full-time tenured professor at the Faculty of Dentistry of McGill University. Dr. Tamimi supervises the design of the clinical trial and statistical analysis, data interpretation.

Dr. Elham Emami is a clinical scientist, with a postgraduate professional training in Prosthodontics (MSc, Université de Montréal), research training in Biomedical Science (Ph.D., Joint program, McGill University & Université de Montréal), Dental Public Health (Postdoctoral fellowship, McGill University), and Cancer Epidemiology (Postdoctoral fellowship,

Environmental Epidemiology and Population Health research group, Université de Montréal). Dr. Emami supervises the design of the clinical trial and statistical analysis, data interpretation.

Dr. Ana Velly, Ph.D., MSc, DDS is an Associate Professor with the Faculty of Dentistry at McGill University, with a MS in Neurologic Science and a PhD in Public Health with an orientation in Epidemiology from the Université de Montréal, followed by post-doctoral training in epidemiology from the Department of Epidemiology, Biostatistics and Occupational Health, McGill University. She supervises Qiman in designing the study as an expert in public health-epidemiology, and pain clinical research,

Dr. Didem Dagdeviren DMD, M. Sc., Ph.D., is an Assistant Professor and the Director of Oral and Maxillofacial Radiology clinic in the McGill University Faculty of Dentistry. Dr. Dagdeviren will supervise Dr. Qiman Gao for the acquisition of Cone Beam CT scans and image analysis.

Dr. Qiman Gao, BDS, MSc (OMFS), is a Ph.D. student in Dr. Tamimi's lab working on optimizing the Needle-Free device to deliver local anesthesia. She is in charge of the whole design and procedure.

Dr. Zovinar Der Khatchadourian D.D.S., is a fully licensed and experienced dentist practicing in Faculty of Dentistry, McGill University. She is also in charge of the orofacial pain clinic at McGill Dental Clinic. She will supervise Dr. Qiman Gao for operation of anesthesia and examination for this clinical trial at McGill Dental Clinic.

Mr. Karim Menassa is the founder of IDEE International R & D Inc. in 1984 and the founder of Medical International Technologies (MIT Canada) Inc. in 2002. He is the inventor of needle-free jet injector platform for Human and Animal applications. He built production and assembly facility to produce Needle-Free Injectors.

F. Potential benefits and risks to the patients and clinicians

The needle-free device can provide better local anesthesia treatment with many advantages:

1. The diameter of the needle-free jet orifice is around 60um, which overcome the limitation of the metal needle and can provide a pain-free injection.
2. Eliminate all needle-related risks: needle phobia, needle fracture, needle trans-infection, needle injury for clinicians and cost of needle disposal, which would benefit both the patient and the clinicians.

3. There is an expected improvement of the patient's dental health once they have pain free treatment. This study has the potential to benefit all the patients who require dental treatment under anesthesia by providing them pain-free treatment, hence more patients are willing to come to the dental clinic.
4. Reduce drug volume and increase drug diffusion, according to preliminary laboratory study.
5. Reduce the required time for injection by providing a faster injection (0.15s/one injection according to lab experiment) and also reduce the time usually the clinician spends on handling patients fear from the needle.
6. When children were given conventional anesthesia, they always struggle and cry which may cause needle fracture and other dangerous results. The usage of the needle-free device will benefit the patients by having shorter therapeutically time and eliminate the risk of needle fracture.
7. For children who are anxious and uncooperative because of pain. The needle-free device will reduce the need for sedation and straps during treatment which will save both time and costs by reducing the patients' pain level and anxiety level.
8. The risks caused by anesthetic drugs might be reduced because the needle-free anesthesia needs less drug volume. For example, the nerve block local anesthesia will result in a numb feeling on lower lip for a long time, which inducing children to bite their lips and cause injury on lips, while the usage of needle-free infiltration anesthesia with less anesthetic volume and shorter duration can reduce the risk of lips injury.

While there are also risks related to local anesthesia:

1. All the other risks or complication related to dental local anesthesia technique and drugs should be similar to conventional local anesthesia technique since we are targeting the same site and using the same drugs. The complications of needle anesthesia include but not limited to failure of anesthesia, allergy, infection, nerve injury, hematoma, dizziness, tachycardia, agitation, nausea, tremor, temporary nerve paralysis, trismus, visual disturbance [29].
2. The previous study showed that some other needle-free device could cause tissue necrosis by increasing the injection site abscesses when arcanobacterium pyogenes is present on the skin surface [16]. The risk in our study will be minimized by the following intervention: First, both the device and the surface mucosa will be sterile; the device will be sterile by autoclave,

and the patients will receive mouth wash before any intervention, and the injection site will be sterile with alcohol swap as part of the standard operation protocol. Second, the Meso-Jet could minimize the risk of tissue necrosis by reducing the risk of push too much bacteria on the skin/mucosa surface into tissue by the “LOW-PRESSURE SYSTEM”, which is a system with high impact to open a micro-wound on the surface, then with low and safe pressure to deliver drug inside the tissue. Until now, there is no report about the necrosis caused by the needle-free device in MIT CANADA. Third, the tissue necrosis is reversible; if this situation happens, we will prescribe local anti-bacteria drug to treat the patient.

3. The needle-free device could be used in other dental applications. Improper use of injector can cause the local anesthetic to be splashed, or target to the wrong site, therefore, hurt patients or dentists. To prevent accidents, dentists and patients should wear protective glasses.
4. In the short term, there might be a possible reversible risk of mucosa ulcer or bleeding due to high pressure, while we will use low pressure which has been confirmed to be safe to minimize these risks. Preliminary lab experiment has shown that this device is safe and the pressure is below the risking pressure that causes nerve damage.

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CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board (IRB) is a registered University IRB working under the published guidelines of the Tri-Council Policy Statement, in compliance with the Plan d'action ministériel en éthique de la recherche et en intégrité scientifique (MSSS, 1998), and the Food and Drugs Act (17 June 2001); and acts in accordance with the U.S. Code of Federal Regulations that govern research on human subjects. The IRB working procedures are consistent with internationally accepted principles of Good Clinical Practices.

At a Board meeting on 10 September 2018, the Faculty of Medicine Institutional Review Board, consisting of:

Patricia Dobkin, PhD	Carolyn Ells, PhD
Catherine Lecompte	Sally Mann. M.S.
Kathleen Montpetit, MSc	Roberta Palmour, PhD
Alexandra Pasca, LL.M.	Margaret Swaine, BA

Examined the research project **A09-M36-18A** titled: *The application of needle-free device on dental anesthesia: a pilot study*

As proposed by: Dr. Faleh Tamimi (Applicant) to _____ (Granting Agency, if any)

And consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

03 April 2019
Date

Chair, IRB

Dean/Associate Dean Faculty

Institutional Review Board Assurance Number: FWA 00004545

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03 April 2019

Dr. Faleh Tamimi
Faculty of Dentistry
Strathcona Anatomy & Dentistry Building
3640 University, Room M60
Montreal QC H2X O3A

RE: **IRB Study Number A09-M36-18A**
The application of needle-free device on dental anesthesia: a pilot study

Dear Dr. Tamimi,

Thank you for responding to the IRB's correspondence concerning the 10 September 2018 full Board review of the above-referenced study. This study was reviewed on behalf of your Doctoral student, Qiman Gao.

The submitted response and revisions are acceptable. This study has received final ethics approval on 03 April 2019:

- Amended Research Protocol, IRB dated March 2019;
- Executive Description for Clinical Operation of Needle-Free Anesthesia (IRB dated March 2019);
- Recruitment Advertisements (text and flyer formats) – *N.B., note the text correction in the first sentence of the text and make the appropriate correction before posting the recruitment material*;
- Questionnaire for Participants (IRB dated March 2019);
- Research Participant Informed Consent Form, Version 2.0, April 2019.

The ethics approval for this study is valid until **September 2019**. The Certificate of Ethical Acceptability is enclosed.

All research involving human subjects is required to undergo an annual ethics review as stipulated in Federal and Provincial documents guiding and regulating research involving human subjects. This annual review is scheduled according to the date of initial approval, and it is the responsibility of the investigator to submit a completed application form for Continuing Ethics Review to the IRB prior to the stop date of the study's ethics approval. A copy of the Continuing Review form is available on the IRB website at: http://www.mcgill.ca/medresearch/ethics/.

The Investigator is reminded of the requirement to report all IRB approved study documents to the

Research Ethics Offices (REOs) for the participating study sites, if applicable. Please contact the individual REOs for instructions on how to proceed. Research funds may be withheld, and/or the study's data may be revoked for failing to comply with this requirement.

Any modifications or unanticipated developments that may occur to the study prior to the annual review must be promptly reported to the IRB. Study modifications cannot be implemented prior to ethics review and approval of the change.

The IRB has assigned this study the following IRB Study Number: A09-M36-18A. Please reference this number for all correspondence with our office.

Regards,

Carolyn Ells, PhD
Co-Chair
Institutional Review Board

Cc: Qiman Gao
A09-M36-18A

QUESTIONNAIRE FOR PARTICIPANTS

Number of Participant:

Operator: Date: / /
(dd/mm/year)

---------- 0- For Assistant: Dental Pulp Test Baseline----------

Tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
Result														
Tooth	47	46	45	44	43	42	41	31	32	33	34	35	36	37
Result														

(e.g. : a. infiltration at 12: test 11 to 13 and 22, b. mandibular inferior alveolar nerve block at left mandible: test 46/47, and 35-37 , c. mental nerve block at right mandible: test 34, 35 and 43-45)

---------- 0- Checklist For Assistant----------

□ Consent form signed

□ Regular oral examination; oral health problem: _____

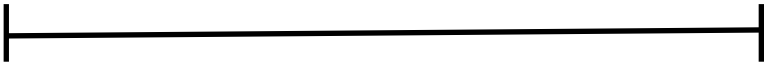
□ Receipt signed

□ Picture took after injection

1st injection □ Left □ Right
Type of anesthesia □ Infiltration; □ MIA NB; □ Mental NB; □ Infraorbital NB;
Injection method □ needle □ needle-free; □ NF-parallel □ NF-vertical;

----------1-For Participants: Before injection----------

Please draw a **vertical line** in the picture bellow to describe your **Pain Level** before the anesthesia.



0
Not pain at all

10
Extremely Pain

Please draw a **vertical line** in the picture bellow to describe your **Anxious Level** before the anesthesia.

0
Not at all
Anxious

10
Extremely
Anxious

----------2- For Participants: 20 second after injection----------

Please draw a **vertical line** in the picture bellow to describe your **Pain Level When receiving** the anesthesia.

0
Not pain
at all

10
Extremely
Pain

Please draw a **vertical line** in the picture bellow to describe your **Anxious Level When receiving** the anesthesia.

0
Not at all
Anxious

10
Extremely
Anxious

----------3 For Assistant - During the injection ----------

1. Time perform injection: _____(h): _____(min): _____(sec)

2. Time the numbness starts: _____(h): _____(min): _____(sec)

3. Time to achieve fully numbness: _____(h): _____(min): _____(sec)

Perform dental pulp test

4. Time the numbness end: _____(h): _____(min): _____(sec)

Initial time of the numbness: min sec

Time to Fully numbness: min sec

Duration of this anesthesia: min sec

----------4- For Assistant Dental Pulp Test----------

Tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
Result														
Tooth	47	46	45	44	43	42	41	31	32	33	34	35	36	37
Result														

(e.g. : a. infiltration at 12: test 11 to 13 and 22, b. mandibular inferior alveolar nerve block at left mandible: test 46/47, and 35-37 , c. mental nerve block at right mandible: test 34, 35 and 43-45)

----------5- For Participants- Questions after injection ----------

1. How do you like the taste?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

2. Is there any other feeling or experience or comments about this anesthesia?

Answer:

.

.

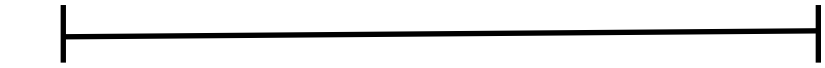
.

2nd injection □ Left □ Right

Type of anesthesia □ Infiltration; □ MIA NB; □ Mental NB; □ Infraorbital NB;
Injection method □ needle □ needle-free; □ NF-parallel □ NF-vertical;

----------1-For Participants Before injection----------

Please draw a **vertical line** in the picture bellow to describe your **Pain Level** before the anesthesia.



0
Not pain at all

10
Extremely Pain

Please draw a **vertical line** in the picture bellow to describe your **Anxious Level** before the anesthesia.

0
Not at all Anxious

10
Extremely Anxious

----------2- For Participants 20 second after injection----------

Please draw a **vertical line** in the picture bellow to describe your **Pain Level When receiving** the anesthesia.

0
Not pain at all

10
Extremely Pain

Please draw a **vertical line** in the picture bellow to describe your **Anxious Level When receiving** the anesthesia.

0
Not at all Anxious

10
Extremely Anxious

----------3 For Assistant - During the injection ----------

1. Time perform injection: _____(h): _____(min): _____(sec)

2. Time the numbness start: _____(h): _____(min): _____(sec)

3. Time to achieve fully numbness: _____(h): _____(min): _____(sec)

Perform dental pulp test

4. Time the numbness end: _____(h): _____(min): _____(sec)

Initial time of the numbness: min sec

Time to Fully numbness: min sec

Duration of this anesthesia: min sec

----------4- For Assistant Dental Pulp Test----------

Tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
Result														
Tooth	47	46	45	44	43	42	41	31	32	33	34	35	36	37
Result														

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----------5- For Participants- Questions after injection ----------

1. How do you like the taste?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

2. Is there any other feeling or experience or comments about this anesthesia?

Answer:

.

.

.

3 After experiencing both needle-free anesthesia and needle anesthesia, what's your preference if you are visiting a dental clinic? Why?

a. needle b. needle-free c. both.

Reason:

----------Before leaving ----------

Did the participant experience any complication bellow?

Left:

□ Bleeding □ Failure of anesthesia □ Allergy
□ Hematoma □ Dizziness □ Tachycardia □ Agitation
□ Nausea □ Tremor □Trismus
□ others: _____ □ No complications

Right:

□ Bleeding □ Failure of anesthesia □ Allergy
□ Hematoma □ Dizziness □ Tachycardia □ Agitation
□ Nausea □ Tremor □Trismus
□ others: _____ □ No complications

----------Follow up ----------

(Complication within 1 week)

Date of phone call or email:

Result:

□ Ulcer □ Infection □ Nerve injury □ Hematoma
□ Allergy □ Temporary nerve paralysis □Trismus
□ Temporary nerve paralysis □ Nerve injury
□ others: _____