Clinical Trial Protocol
Iranian Registry of Clinical Trials

02 Jun 2026

Antibacterial effects of elastomeric ligatures coated with Kombucha-synthesized bacterial nanocellulose against streptococcus mutans Randomized Clinical

Protocol summary

Study aim

Determination the Antibacterial effects of elastomeric ligatures coated with Kombucha-synthesized bacterial nanocellulose against streptococcus mutans

Design

A three-way blind clinical trial with a block method and a control group, the sample size in each group was calculated to be 36. A total of 72 patients between 15 and 30 years of age

Settings and conduct

A three-way blind clinical trial with the قشدیخئهظشفهخد block method. Among the people who refer to the Department of Orthodontics, Faculty of Dentistry, Shiraz University of Medical Sciences, who need permanent orthodontic treatment.

Participants/Inclusion and exclusion criteria

Male and female؛Age ۱۵ to ۳۰ years / non pregnant؛No significant medical history or drug use؛No anti-inflammatory or antibiotic medications taken within 3 months prior to the study؛habit of brushing twice daily with fluoride toothpaste؛No history of periodontal therapy؛Probing depth less than 4 mm؛No gingivitis or active carious lesion؛Gingival and plaque index value <۱ Diseases and medication affect dental biofilm؛Pregnancy؛Poor oral hygiene؛Active periodontal disease؛Severe crowding of anterior teeth or malposition of the lateral incisor؛Smoking؛History of mouth breathing

Intervention groups

A type of brackets , composite and adhesive is used for bonding in all patients. After the initial sorting and place of placing the 18 steel wire, the basic saliva sample is collected from the intervention and control group patients. The dental plaque sample (T0) is taken from the upper lateral incisors bilaterally using a sterile periodontal probe. PEPs obtained plaque samples at T0, BNC release ligatures were used for experimental group patients and conventional ligatures were used for control patients. Saliva and plaque samples were collected again at 7, 14, 21, and 28 days after placement of ligatures is collected.

Main outcome variables

Bacterial colony counting

General information

Reason for update

Acronym

bacterial nanocellulose = BNC

IRCT registration information

IRCT registration number: **IRCT20181121041713N5**
Registration date: **2023-10-22, 1402/07/30**
Registration timing: **prospective**

Last update: **2023-10-22, 1402/07/30**
Update count: **0**

Registration date

2023-10-22, 1402/07/30

Registrant information

Name

neda babanouri

Name of organization / entity

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Iran (Islamic Republic of)

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Recruitment status

Recruitment complete

Funding source

Expected recruitment start date

2023-10-28, 1402/08/06

Expected recruitment end date

2023-11-25, 1402/09/04

Actual recruitment start date

empty

Actual recruitment end date

empty

Trial completion date

empty

Scientific title

Antibacterial effects of elastomeric ligatures coated with Kombucha-synthesized bacterial nanocellulose against streptococcus mutans Randomized Clinical

Public title

Antibacterial effects of elastomeric ligatures coated with bacterial nanocellulose

Purpose

Diagnostic

Inclusion/Exclusion criteria

Inclusion criteria:

Male and female patients Age 15 to 30 years non pregnant No significant medical history or drug use No anti-inflammatory or antibiotic medications taken within ۳ months prior to the study No chewing gum or mouthwash use in the week before and during the study habit of brushing twice daily with fluoride toothpaste No history of periodontal therapy or current periodontal disease Probing depth less than ۴ mm across the entire dentition No gingivitis or active carious lesion Gingival index and plaque index (Silness-Löe) value <1

Exclusion criteria:

Diseases and medication use likely to affect dental biofilm Pregnancy Poor oral hygiene Active periodontal disease Severe crowding of anterior teeth or malposition of the lateral incisor likely to interfere with the cleaning of the tooth surface Smoking History of mouth breathing

Age

From **15 years** old to **30 years** old

Gender

Both

Phase

3

Groups that have been masked

- Participant
- Care provider
- Data analyser

Sample size

Target sample size: **72**

Randomization (investigator's opinion)

Randomized

Randomization description

The selected 72 patients will be randomly divided into test and control groups (n=36). Block randomization method will be used with block size of 6, used online software RANDOM.ORG to allocate patients to intervention and control groups with a ۱:۱ ratio. Subsequently, the random sequences to the study groups will be concealed in opaque envelopes and shuffled before the intervention to increase the unpredictability of the random allocation sequence. Each patient will be asked to pick a sealed envelope to assign to either test or control .Allocation concealment will be done to avoid selection bias.

Blinding (investigator's opinion)

Triple blinded

Blinding description

triple-blind clinical trial Due to the color change in elastomeric ligatures from gray to gold following BNC coating, the clinician could not be blind to the study groups. However, the patients, the assessor who collect the biofilm samples and the statistician will be blind to the study groups

Placebo

Used

Assignment

Parallel

Other design features

Secondary Ids

empty

Ethics committees

1

Ethics committee

Name of ethics committee

Ethics committee of Shiraz University of Dentistry

Street address

Mehr St.- Qasr Al-Dasht St.- Faculty of Dentistry

City

Shiraz

Province

Fars

Postal code

7195615878

Approval date

2023-08-13, 1402/05/22

Ethics committee reference number

IR.SUMS.DENTAL.REC.1402.055

Health conditions studied

1

Description of health condition studied

Antibacterial effects of elastomeric ligatures coated with Kombucha-synthesized bacterial nanocellulose against streptococcus mutans

ICD-10 code

ICD-10 code description

Primary outcomes

1

Description

Bacterial colony counting

Timepoint

The beginning of the study and days 7, 14, 21 and 28

Method of measurement

Counting the number of bacterial colonies after sampling

Secondary outcomes

empty

Intervention groups

1

Description

Intervention group: A minimum of ۲۰ brackets will be used for patients. fixed pre adjusted edgewise appliances (Mini Master Bracket, ۰.۰۲۲-in MBT prescription; American Orthodontics, USA) with a slot size of ۰.۰۲۲×۰.۰۲۸ inch will be used in all patients. The same type of composite and adhesive will be used for bracket bonding in all patients. After primary leveling and alignment and at placing of steel wire ۱۸, baseline saliva samples will be collected.ntervention group receive coated elastomeric ligatures.Dental plaque sample (T۰) will be obtained from the upper lateral incisor site bilaterally using a sterile periodontal probe. manually delete the supra-gingival plaque , dries and then inter the sterile paper points with tweezers in selected sites. The tip left in situ for about one minute , then removed and inserted into a sterile container for sending in the laboratory (۱۵). The plaque sample will be transferred into a microtube containing ۱ mL saline. After obtaining the plaque samples in T۰, BNC releasing ligatures will be used for patients in the test group while conventional ligatures will be used for control patients. The samples will be placed next to dry ice and will be sent to a microbiology lab in less than ۲ h. Saliva and plaque samples will be collected again at ۷, ۱۴, ۲۱ and ۲۸ days after placement of ligatures (T۱, T۲, T۳, T۴)Preparation of coating solution and Preparation of elastomeric ligatures:The Kombucha SCOBY starter culture was obtained from an Iranian origin and activated every ۱۴ days. The raw cellulose material in the study was Kombucha membranes, an outgrowth of a symbiotic culture of bacteria and yeast fermenting tea broth. The process of making tea infusion involved steeping ۵ grams of green tea (Basilur green tea, Ceylon) in boiling sterile water for ۱۵ min followed by adding ۱۲% high fructose corn syrup as sweetener (Hungrasweet F۵۰, HungranaKft., Ipartelep, Hungary). The sweetened infusion, amounting of two liters, was aseptically poured into a ۳-liter sterile brown glass bottle. Then, ۱۰% of Kombucha tea previously fermented was added as inoculum. The symbiotic culture of bacteria and yeast (SCOBY), originating from a Romanian culture, contained acetobacteria from Komagataeibacter and Gluconobacter genera, as well as yeasts from several genera such as Zygosaccharomyces, Brettanomyces/Dekkera and Pichia, and lactobacteria. A cotton towel was wrapped around the brown glass bottle and secured at the neck of the bottle. The Kombucha culture was allowed to ferment at around ۲۳ °C for ۳۰ days in order to yield Kombucha vinegar. The initial brown Kombucha membranes, referred to as K_۰ sample, weighed ۴۶۱ g. NaOH was tested in ۱ and ۴ M concentrations (۱:۲ solid:liquid ratio) to extract KM from proteins, saccharides, and amino acids, resulting in samples encoded K_۱M and K_۴M. With respect to the thickness of initial membranes, they were washed between ۱۰ to ۳۰ times (۱۰ min each time) by using ۱ M NaOH solutions. The washing process was made more effective by using an Elmasonic P ultrasonic bath (Elma, Singen, Germany). Following the alkaline treatment and ultrasonication process, the purified KMs underwent multiple washes with distilled water until reaching a neutral pH. The beige white membranes (K_۱M), weighing ۳۴۷ g, underwent additional treatment with ۴ M NaOH before being neutralized with distilled water to produce K_۴M. Purified K_۴M never-dried membranes, weighing ۳۳۰ g (۱% w dried cellulose, all concentrations will refer to dry-cellulose content), were ground with a blender (۱۰۰۰ W, ۱۰ series of ۵ min) and diluted with ۵.۵ L water (۵.۷ × ۱۰-۲% w/v) to produce the K_B samples. The K_B samples were subjected to ۳ hours of wet deep grinding by a recirculating colloidal mill (۲۵۰ µm blade space and ۲۵ L/min flow rate), representing about ۸۰۰ passes. Then, the following approach weas experimented:The colloidal mill sample was diluted ۱۰× (۵.۷ × ۱۰-۳% w/v) and then atomized (or spray-dried) by using a Mini Spray dryer B-۲۹۰ (Buchi, Flawil, Switzerland) to attain BNC in dried form. The dried form of nanocellulose is crucial for various bionanocomposites. The optimized operating parameters for nano-atomization were a flow rate of ۴ mL/min for the cellulose suspension, an air flow of ۵۰۰ L/h, with inlet and outlet temperatures of respectively ۱۷۵ °C and ۹۰ °C

Category

Prevention

2

Description

Control group: A minimum of ۲۰ brackets will be used for patients. fixed pre adjusted edgewise appliances (Mini Master Bracket, ۰.۰۲۲-in MBT prescription; American Orthodontics, USA) with a slot size of ۰.۰۲۲×۰.۰۲۸ inch will be used in all patients. The same type of composite and adhesive will be used for bracket bonding in all patients. After primary leveling and alignment and at placing of steel wire ۱۸, baseline saliva samples will be collected from both the control group receive non coated elastomeric ligatures

Category

Placebo

Recruitment centers

1

Recruitment center

Name of recruitment center

Orthodontic Department of Shiraz Dental Faculty

Full name of responsible person

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Sponsors / Funding sources

1

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Grant name
Grant code / Reference number
Is the source of funding the same sponsor organization/entity?
Yes
Title of funding source
Shiraz University of Medical Sciences
Proportion provided by this source
100
Public or private sector
Public
Domestic or foreign origin
Domestic
Category of foreign source of funding
empty
Country of origin
Type of organization providing the funding
Academic

Person responsible for general inquiries

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Sharing plan

Deidentified Individual Participant Data Set (IPD)
Yes - There is a plan to make this available
Study Protocol
Yes - There is a plan to make this available
Statistical Analysis Plan
Yes - There is a plan to make this available
Informed Consent Form
Yes - There is a plan to make this available
Clinical Study Report
Yes - There is a plan to make this available
Analytic Code
Yes - There is a plan to make this available
Data Dictionary
Yes - There is a plan to make this available
Title and more details about the data/document
General data including age, sex, medical and dental status, along with outcomes and results can be shared.
When the data will become available and for how long
Access starts 6 months after results are published
To whom data/document is available
Researchers working in academic and scientific institutions
Under which criteria data/document could be used
Access for researchers and people who intend to continue scientific research on this matter.
From where data/document is obtainable
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What processes are involved for a request to access data/document
The information will reach them within one month at the latest after the request and review of the request.
Comments