**Study Protocol**

# Study Title: Effect of Local Application of Hyaluronic Acid on Wound Healing after Crown Lengthening Procedure in Smokers: A Randomized Controlled Clinical Trial, Double Blind

**Protocol No.:** E-20-4826

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## List of Abbreviations

| Abbreviation | Full Term |
|---|---|
| BoP | Bleeding on Probing |
| CI | Confidence Interval |
| CL | Crown Lengthening |
| CONSORT | Consolidated Standards of Reporting Trials |
| DNA | Deoxyribonucleic Acid |
| GCP | Good Clinical Practice |
| GI | Gingival Index |
| HA | Hyaluronic Acid |
| ICH-GCP | International Council for Harmonisation - Good Clinical Practice |
| IRB | Institutional Review Board |
| ITT | Intent-to-Treat |
| PI | Plaque Index |
| PP | Per-Protocol |
| PPD | Periodontal Probing Depth |
| SPSS | Statistical Package for the Social Sciences |
| VAS | Visual Analogue Scale |

## 1. Background and Rationale

Periodontal surgical procedures such as crown lengthening (CL) require optimal wound healing for functional and esthetic success. Smoking impairs healing, posing a significant challenge to post-surgical outcomes (1, 2). Hyaluronic acid (HA), a naturally occurring glycosaminoglycan, is integral to tissue repair and has anti-inflammatory, bacteriostatic, and pro-angiogenic properties (3, 4). In periodontal therapy, HA has shown potential to accelerate wound healing, reduce post-operative discomfort, and improve clinical outcomes (5–7). Several randomized controlled trials and systematic reviews have highlighted the efficacy of HA in both surgical and non-surgical periodontal procedures (6–9). However, limited evidence exists on the effectiveness of HA in enhancing healing, specifically following CL in smokers, a subgroup with compromised periodontal healing potential (2, 10). This study aims to assess the clinical effectiveness of local HA application during osseous crown lengthening procedures in smokers by evaluating post-operative healing, pain, and clinical periodontal parameters.

## 2. Study Objectives

### 2.1. Primary Objective

To evaluate the effect of locally applied cross-linked HA on soft tissue healing after osseous CL in smokers, assessed using the Landry Healing Index at 2 and 6 weeks postoperatively.

### 2.2. Secondary Objectives

In addition to the primary objective, the study seeks to explore several clinically relevant secondary endpoints:

- To compare post-operative pain using the Visual Analogue Scale (VAS)
- To assess periodontal parameters: probing pocket depth (PPD), bleeding on probing (BoP), plaque index (PI), and gingival index (GI)
- To evaluate radiographic bone level stability

## 3. Study Design

This is a randomized, double-blind, parallel-group, controlled clinical trial. Participants will be randomly allocated to either the test group (HA application) or the control group (saline application) in a 1:1 ratio. The study will be conducted at the Department of Periodontics, College of Dentistry, King Saud University, Riyadh, Saudi Arabia.

## 4. Sample Size and Randomization

Based on an α error of 0.05 and 80% power, 15 patients per group are required. A total of 30 participants will be recruited, considering a 15% allowance for dropouts. Randomization will be performed using a computer-generated list (SPSS v22.0), and allocation will be concealed. Both the participant and the outcome assessor will be blinded to the intervention.

## 5. Eligibility Criteria

The eligibility of participants is defined by a set of clearly outlined inclusion and exclusion criteria to ensure homogeneity of the study sample and to safeguard participant safety.

### 5.1. Inclusion Criteria

- Healthy adult smokers (≥18 years)
- Referred for crown lengthening
- Willingness to provide informed consent

### 5.2. Exclusion Criteria

- Uncontrolled systemic diseases (e.g., diabetes with HbA1c >7%, osteoporosis)
- Pregnancy or lactation
- Use of medications affecting bone/mucosal healing (e.g., steroids, antiresorptive therapy)
- Antibiotic use in past 2 months
- Restorations obstructing PPD measurements
- Active periodontal disease
- Inability to consent

Smokers are defined as individuals smoking ≥10 cigarettes/day.

## 6. Interventions

- **Test Group (CL + HA):** CL followed by application of cross-linked HA gel (hyaDENT BG, Bioscience, Germany) on the root surface using a cartridge syringe with 23G needle, as per manufacturer's instructions.
- **Control Group (CL + Saline):** CL followed by saline irrigation (placebo).

In both groups, the flap will be repositioned and sutured using 4/0 silk. Postoperative instructions include:

- No brushing/flossing for 3 days
- Soft diet for one week
- Saline gauze cleaning from Day 4 onward

## 7. Follow-Up and Assessments

**Timepoints:** Baseline, 2 weeks, and 6 weeks

**Clinical Parameters:**

- **PPD:** Measured at six sites per tooth using a Hu-Friedy probe
- **BoP:** Presence/absence of bleeding post-probing
- **PI:** Presence/absence of plaque on four surfaces using disclosing solution
- **GI:** Assessed using Löe and Silness criteria
- **Healing Index:** Landry's scale (1–5)
- **Pain:** VAS (0=no pain, 10=worst pain)

**Radiographic Assessment:** Standardized vertical bitewing radiographs at baseline and 6 weeks.

**Microbiological Samples:** Plaque and saliva collected and stored in Eppendorf tubes; DNA extracted and analyzed for specific bacterial species (e.g., *A. actinomycetemcomitans*, *P. intermedia*).

## 8. Data Collection and Management

Data will be recorded in standardized forms and entered into a secure database. Data will be anonymized and maintained for at least 5 years. Double data entry and validation will be performed. The PI is responsible for data quality and integrity.

## 9. Statistical Analysis

Statistical analysis is described in a separate Statistical Analysis Plan. In brief:

- **Primary outcome:** Compared using the Mann-Whitney U test
- **Secondary outcomes:** Parametric and non-parametric tests based on data distribution

- **Software:** SPSS v24.0

## 10. Ethical Considerations

The study was approved by the IRB at King Saud University (Ref. No. 20/0416/IRB). It adheres to the Declaration of Helsinki and ICH-GCP guidelines. Written informed consent will be obtained from all participants using the approved form (KSU-IRB 005-E). Participants may withdraw at any time without penalty. Adverse events, if any, will be recorded and managed appropriately.

## 11. Dissemination Plan

Study results will be published in peer-reviewed journals and presented at conferences. Data will be reported in line with CONSORT guidelines.

## 12. References


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