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IRAS ID: 316275 V.2 04/05/2023

Effectiveness of two bone substitutes, natural and synthetic, in preserving the alveolar ridge of single-rooted teeth: a pilot single-blind, parallel randomised controlled trial

Date: 04/05/2023

TITLE:	**Effectiveness of two bone substitutes, natural and synthetic, in preserving the alveolar ridge of single-rooted teeth: a pilot single-blind, parallel randomised controlled trial**
ACRONYM:	**BSNS (Bone Substitutes Natural Synthetic)**
Protocol version:	**1.0**
Date:	**15.11.2022**
Reviewed version	**2**
Date:	**04.05.2023**
IRAS project ID	
Trial registration	**TBC**
Study design	**Pilot** single-blind, parallel randomised controlled trial
Funding and support	**None**
Project CODE	
Confidentiality	**This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigators Team, host organisation (s), and the Research Ethics Committee members unless authorised to do so.**

Contents

KEY STUDY CONTACTS

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Joint-sponsor(s)/co-sponsor(s)	n/a
Funder(s)	n/a
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Study summary

Study title	Effectiveness of bone substitutes, natural and synthetic in preserving the alveolar ridge of single-rooted teeth: a pilot single-blind, parallel randomised controlled trial
Internal ref.no. (or short title)	BSNS (Bone Substitutes Natural vs Synthetic)
Study design	Pilot single-blind, parallel randomised controlled trial
Study participants	Adult patients who require extraction of single-rooted teeth
Planned size of Sample	34 patients, 2-4 dental practices
Planned study Period	12 months with 24-week follow-up
Research Questions/Aim(s)	**Research questions**: What are the differences between natural and synthetic

	bone substitutes in preserving the alveolar ridge dimensions based on: i. Changes in the width of the alveolar ridge (expressed in millimetres) from baseline to 24-week follow-up? ii. Changes in height of the alveolar ridge (expressed in millimetres) from baseline to 24-week follow-up? The two changes will be assessed with either an intraoral scanner or impressions and clinical photographs. iii. Changes in the vertical crestal bone level assessed in millimetres at 24-week follow-up? iv. Presence of bone around the alveolar ridge to be assessed using biopsy (up to 3mm diameter by 8mm length) for histology (qualitative assessment of bone infiltration) and PCR to determine the concentration (in nanograms) of gene expression for bone markers at 24-week follow-up? **Aim**: To investigate the effectiveness of two low substitution rate bone substitutes, natural and synthetic in preserving the alveolar ridge of single- rooted teeth following extraction.

FUNDING AND SUPPORT IN KIND

FUNDER(S)	n/a

ROLE OF STUDY SPONSOR AND FUNDER

The sponsor, UCLan, takes on overall responsibility for proportionate, effective arrangements being in place to set up, run and report a research project

Study coordination

Dr Fadi N Barrak, the Principal investigator (PI), will be responsible for researching the site under the Chief investigator's supervision

Lay Summary

After a tooth is extracted, the bone that supports and surrounds the tooth can considerably shrink. This loss of bone volume around the missing tooth can make future treatment with dentures or dental implants more challenging. It may even necessitate surgical procedures to increase the bone volume in the area where the extraction was carried out and replace the lost bone. Therefore, it is vital to consider the preservation of the bone that supports the tooth to limit the reduction of bone volume after an extraction. Bone substitutes can be used to pack the extraction socket to preserve the space.

The most commonly used bone substitutes in dentistry are cow bone. However, as people are becoming less inclined to use animal derivates, synthetic bone substitutes could become a feasible alternative. This study aims to evaluate the efficacy of one of these synthetic bone substitutes – bioactive glass (BAG) – in preserving the bone that supports a tooth after an extraction.

A few synthetic alternatives to cow bone are already available on the market. However, their properties are not as good as traditional cow bone. They are resorbed much more quickly, and therefore, in the long term, they fail to maintain the shape and volume of the bone in the extraction socket. What is different about BAG is the fact that it not only has similar long-term preservation features as cow bone, but it also has the potential to surpass cow bone because of its unique antibacterial activity.

The study will be conducted at the University of Central Lancashire, Imperial College London and dental clinics in Midlands, Southeast and Northwest England. We will recruit 34 patients who require tooth extractions and divide them into two equal groups. Patients in group A will receive BAG substitute, while those in the group B will receive the natural bone substitute. For both groups, the bone substitute will be inserted immediately after an extraction, and the socket will be sealed with a membrane sutured in place. Patients in both groups will be reviewed after 2 weeks to remove the suture. Patients will have another review at 24 weeks, during which the researcher will take a scan or mould, a photograph, and a small biopsy sample of the area where the bone substitute was placed. Measurements obtained at the 24-week follow-up will be used to evaluate the effectiveness and differences between natural and synthetic bone substitutes in preserving the bone supporting a tooth after an extraction.

The benefit of this study lies in the possibility of having a synthetic and antibacterial alternative to a naturally derived bone substitute. The results will inform the design of a full randomised controlled trial.

Abstract

Introduction

Using bone substitutes to preserve the alveolar ridge of a missing tooth is important for ensuring the retention and stability of the prosthesis used as a replacement. In dentistry, the most used bone substitutes are bovine derived. However, the movement toward a more environmentally accountable society has created a need for synthetic bone substitutes which are not derived from animals. Therefore, this study aims to evaluate the effectiveness of a bovine-derived bone substitute and bioactive glass (BAG) derived bone substitute in preserving the alveolar bone of a tooth after an extraction.

Methods

A pilot single-blind two-arm parallel randomised control trial will be conducted in dental clinics in the Midlands, Southeast and Northwest England. A total of 34 adult patients, who require the extraction of a single-rooted tooth, will be randomly assigned to experimental and control group at a 1:1 ratio.

After tooth extraction, the bone substitute will be introduced into the socket; the control group A will receive the bovine-derived bone the intervention group B the bioactive glass derived bone. All patients will be reviewed after 2 weeks for suture removal. Patients' follow-up will be 24 weeks after the procedure. Outcome data will be collected at baseline and at 24 weeks. The primary outcome is the effectiveness of bioactive glass derived bone in preserving the alveolar ridge dimensions compared to bovine-derived bone. This will be assessed by measuring changes in the width of the alveolar ridge from baseline (augmentation) to 24-week post augmentation using a linear regression model including baseline measurements as covariates. Secondary outcomes are:

- Changes in the height of the alveolar ridge from augmentation to 24-week post augmentation
- Changes in vertical crestal bone level at 24-week post augmentation
- Presence of bone markers at 24-week post augmentation
- Qualitative Bone infiltration at 24-week post augmentation

Discussion

The results of this pilot study will inform the design and implementation of a full scale randomised controlled trial to investigate the effectiveness of bioactive glass derived bone compared with bovine derived bone in preserving the alveolar bone of a tooth after an extraction.

Ethics and dissemination

Ethics approval was obtained from the University of Central Lancashire xxxxxxx. Results will be published in peer-review and professional journals, presented at scientific conferences, and disseminated to service users and their families via media.

Trial registration number: XXXXX

Effectiveness of two bone substitutes, natural and synthetic, in preserving the alveolar ridge of single-rooted teeth: a single-blind, parallel randomised controlled trial

Background

What is the problem to be addressed?

Implant dentistry has expanded dramatically over the last decade globally and is forecast to continue to do so for the foreseeable future, not least due to the increasing proportion of over 60-year-olds within the population. For example, a study conducted in 2018 reported that the prevalence of dental implants in the United States increased from 0.7% in 1999 to 5.7% in 2015(1) . Elani et al. (1) also noted that the highest absolute growth in prevalence (12.9%) was among the age group 65 to 74 years. Implant treatments are varied and approximately 50% involve the use of bone substitutes to compensate for the jawbone deficiencies and enable implant fixtures' placement (2,3). The use of bone substitutes has grown in parallel with implants. These substitutes are used routinely for 'guided bone regeneration', sinus augmentation, treating peri-implantitis (infection around implants where bone loss occurs), and ridge preservation (4–6). The latter involves placing bone substitute in a fresh extraction socket, immediately after the tooth is removed in order to preserve the jawbone ridge for future implant placement (7,8) .

Bone substitute materials available in the market include allografts (human donated bone), xenografts (from a different species, which can be plant or animal) and alloplasts (synthetic) (6). Historically, the most commonly used material has been xenograft of bovine origin due to its ease of handling and extensive research to support its safety and efficiency in maintaining bone volume. In a 5-year prospective study, Ozkan et al (9). reported that using bovine-derived bone in 1-stage sinus augmentation resulted in sufficient quality and volume of bone for implant placement and a 100% implant survival rate after the follow-up period.

Despite the effectiveness of bovine xenografts, the movement towards a more environmentally accountable society has led to patients finding animal-derived bone substitutes less acceptable (10,11). As clinicians, we also must consider this important aspect of our work and be accountable in terms of our material choices while bearing in mind the environment and patient safety and benefit.

The current synthetic products on the market work well in terms of safety; however, they are mainly calcium phosphate and sulphate based and have a high substitution rate. That means that they are resorbed and remodelled quickly when compared to the bovine bone substitutes

(12) The allografts (human origin) also have a high substitution rate. It is important to have a low substitution rate to maintain the shape and volume of the jaw ridge for the long term to have a stable result.

Bioactive glass synthetic bone substitute (Bonalive®) is a material that has been shown to have a low substitution rate and antibacterial properties. It has been used successfully in the orthopaedics, craniofacial and ear nose and throat (ENT) specialities for the treatment of osteomyelitis, sinusitis and bony cyst cavities (13,14). There have also been a few maxillofacial surgery case reports (15,16) on its usage in the jaws. However, to the best of our knowledge, the applicability of this material in implant dentistry has not been reported in the literature.

Aim

To investigate the effectiveness of two low substitution rate bone substitutes, natural (Bio-Oss®) and synthetic (Bonalive®) in preserving the alveolar ridge of single-rooted teeth following extraction.

Key research questions

Is there a difference between natural (Bio-Oss®) and synthetic (Bonalive®) bone substitutes in preserving the alveolar ridge dimensions based on:

I. Changes in the width of the alveolar ridge (expressed in millimetres) from augmentation (baseline) to 24-week follow-up (post augmentation).

II. Changes in the height of the alveolar ridge (expressed in millimetres) from augmentation (baseline) to 24-week follow-up (post augmentation).

Both changes will be assessed using intra-oral scanners (this does not involve radiographs) or impressions with clinical photographs.

III. Changes in the vertical crestal bone level assessed in millimetres, using the periapical radiographs taken pre-extraction and at 24-week follow-up and intra-oral scanners (this does not involve radiographs) or impressions with clinical photographs

IV. Presence of bone around the alveolar ridge assessed using biopsy (up to 3mm diameter by 8mm length) of the centre of the augmented socket. The biopsy sample will be used for histology (qualitative assessment of bone infiltration) and PCR to determine the concentration (in nanograms) of gene expression for bone markers (including osteopontin, osteocalcin) at 24-week follow-up (post augmentation).

Trial design

It is a pilot single-blind two-arm parallel randomised control trial that aims to assess the differences between two bone substitutes, natural versus synthetic. The study will mimic the routine practice, and patients will be randomly allocated to receive either the natural (control) or synthetic (intervention) bone substitutes. The patient follow-up period will be 24 weeks from the baseline data collection point.

Methods

Study setting

The study will be conducted in dental clinics in the Midlands, Southeast England and Northwest England.

Eligibility criteria for participants

Inclusion criteria

Adult patients (age >18) in need of extraction of a single-rooted tooth attending the clinics selected for the study

Single units in a dentate patient

Non-surgical extraction – no flap raised, and no bone removed

Intact socket walls post-extraction

Bleeding sockets

Stable periodontal health

ASA Class I or II patients. The American Society of Anaesthesiologists (ASA) classification is a system of physical status evaluation developed to offer clinicians a simple categorisation of a patient's physiological status to help predict operative risks (Appendix 1).

Exclusion Criteria

Multiple adjacent extractions

Presence of active periodontal disease

Socket walls not intact - >50% bone loss in any of the four walls

Sclerotic sockets post-extraction – the socket does not fill up with blood post-extraction

Denture wearer – the extraction socket site under the load of a denture

Patients on medication that can affect bone healing e.g., bisphosphonates (oral or intravenous), selective serotonin reuptake inhibitors (SSRI), methotrexate, proton pump inhibitors (PPI)

Uncontrolled diabetes

Smokers

Immunosuppressed Patients

Patients with a history of myocardial Infarction in the last year

ASA Class > II patients (17)(Appendix 1)

Intervention

The test bone substitute is Bonalive®, bioactive glass S53P4, which contains SiO_2, Na_2O, CaO, and P_2O_5 (granule size 0.5-0.8mm) (18) Bonalive® is osteoconductive, meaning that it has the ability of promoting bone growth across the granules and the grafting area and slowly replace it with new bone over time. Bonalive® is osteostimulative and has antibacterial properties (19).

Comparator

The comparator is Bio-Oss®, deproteinized bovine bone granules (granule size 0.25- 1mm) (20). Bio-Oss® is osteoconductive, which means it acts as a scaffold only for new bone to grow (21,22).

Both products have a low substitution rate and are expected to have similar ridge support and shape maintenance capability.

Management of the bone substitutes

The bone substitutes will be used according to the manufacturers' instructions and protocols (Appendix 2 and 3). The materials are supplied in sealed sterile packaging with clearly displayed expiry dates, LOT numbers and CE marks. The materials' LOT numbers and expiry dates will be recorded on the patient records and on a separate 'Surgery Logbook' with an identification number for the patient traceable to the appropriate batch of materials.

The use of the material will follow standard surgical protocols and excess material will be discarded according to clinical waste guidelines.

Clinical procedure

The bone substitute will be used according to the ridge preservation protocol, which will be standardised between the different centres. As part of standard clinical practice, a periapical radiograph will be taken before an extraction. Following the removal of a tooth, the inflamed area of the socket will be curetted to ensure a 'clean' socket. The case will be excluded if:

- The socket walls are very sclerotic with a lack of fresh bleeding, which is essential for healing.
- The socket walls are damaged. The limit is a loss of 50% or more of one of the walls. The assessment of the extraction socket will be done by the clinicians performing the procedures (23).

The bone substitute will then be introduced using a standard sterile protocol, with gentle packing of the material in the sockets to ensure the material reaches the apex of the socket but is not condensed with pressure. The socket will then be sealed with a collagen matrix with non-resorbable sutures. Review appointment for suture removal will be required 2 weeks post-procedure.

Patients will be advised not to fly for two weeks post-procedure or until after the clinicians have reviewed them. Intra-oral scans or impressions with clinical photographs will also be taken on the day the procedure is carried out. The follow-up will take place 24 weeks post-procedure, when a PA radiograph, intra-oral scans or impressions, clinical photographs and a minimally invasive trephine biopsy will be carried out.

Management of tissue samples in the dental clinic

Each sample taken will be split into two segments (one for histology and the other for PCR). The segment for histology will be fixed in formaldehyde, and the one for PCR analyses will be snap-frozen in liquid nitrogen (or dry ice) and placed in -80C (or -20C, in RNAlater®-ICE) freezer for storage. The samples will be transported from the private dental clinics to the Imperial College Healthcare Tissue Bank (ICHTB) as they are collected.

Management of tissue samples for Histology

Packaging of tissue samples for Histology

The packaging will adhere to ADR Packing Instruction 650 for BIOLOGICAL SUBSTANCE, CATEGORY B (Appendix 4). This will consist of three components:

- A primary package which will contain the sample.
- A secondary package which will contain the primary package and sufficient absorbent material to soak up any spillage that occurs.
- A tertiary/outer packaging which will be rigid and have at least one surface with a minimum dimension of 100mm x 100mm

Labelling of tissue samples for Histology

- Each package will be clearly labelled with the delivery address and sender's details. It will also have emergency contact details, including a named person and telephone number for the sender (the private dental clinic) and recipient (ICHTB).
- The outer package will be clearly marked with BIOLOGICAL SUBSTANCE and CATEGORY B letters at least 6mm high. This will be displayed adjacent to the UN3373 label.
- The UN3373 label will be affixed to the outside packaging as a square set at an angle of 45° (e.g., diamond shaped), with each side having a length of at least 50 mm, the width of the line will be at least 2mm and the letters and numbers at least 6mm high (see UN3373 label example in Appendix 5). The background of the mark will have a contrasting colour to the surface of the package.

Transport documentation of tissue samples for Histology

Paperwork with a contents list will be included within the package between the secondary and the outer packaging. The paperwork will be placed in waterproof packaging and, if handwritten, it will be in permanent ink.

Tracking tissue samples for Histology

The management of the tissue samples at ICHTB will be both ethical and compliant with the Human Tissue Authority (HTA) regulations. (See ICHTB's HTA license number in appendix

6). For all samples, a record containing pseudo-anonymised information about the donor, the samples taken, the type of consent obtained, and the surgical procedure used to collect the samples will be created. This information will be recorded in an online database which is compliant with the General Data Protection Registration legislation.

Laboratory processing of tissue samples for Histology

The procedure for processing the sample in the laboratory will be similar to that used in a study by Shi et al. (24). Samples will be dehydrated through a series of increasing concentrations of ethanol and embedded in white acrylic resin for sectioning. The sample blocks will be ground successively with (800, 2000 and 4000 grit) grinding paper to expose and polish the bone/hard tissue. The polished surface will then be glued onto an acrylic disc. The tissue block will then be ground with the same series of grinding paper resulting in a sample of approximately 50-100 µm in thickness. According to the manufacturer's instructions, sections will be stained with Gill's Haematoxylin and Eosin. Stained samples will then be examined and imaged under a microscope.

Management of tissue samples for PCR.

Packaging of tissue samples for PCR

Samples will be placed in a primary package (RNAlater®-ICE in leak-proof Cellstor® biopsy pots) and put in a secondary package (a BiTran® double-zip lock bag), transported in a dry ice-filled styrofoam box. The outer packaging will allow for carbon dioxide gas release to avoid gas accumulation and potential rupturing of packaging or explosion.

Labelling of tissue samples for PCR

- Each package will be clearly labelled with the delivery address and sender's details. It will also have emergency contact details, including a named person and telephone number for where the package is being sent from and where it is going.
- A Class 9 - Miscellaneous Dangerous Goods label with the words DRY ICE next to it, will be clearly visible on the outside packaging. (See UN1845 label example in Appendix 7).

Transport documentation and tracking tissue samples for PCR

The protocols for transport documentation and tracking of the tissue samples will be the same as that for samples taken for histology.

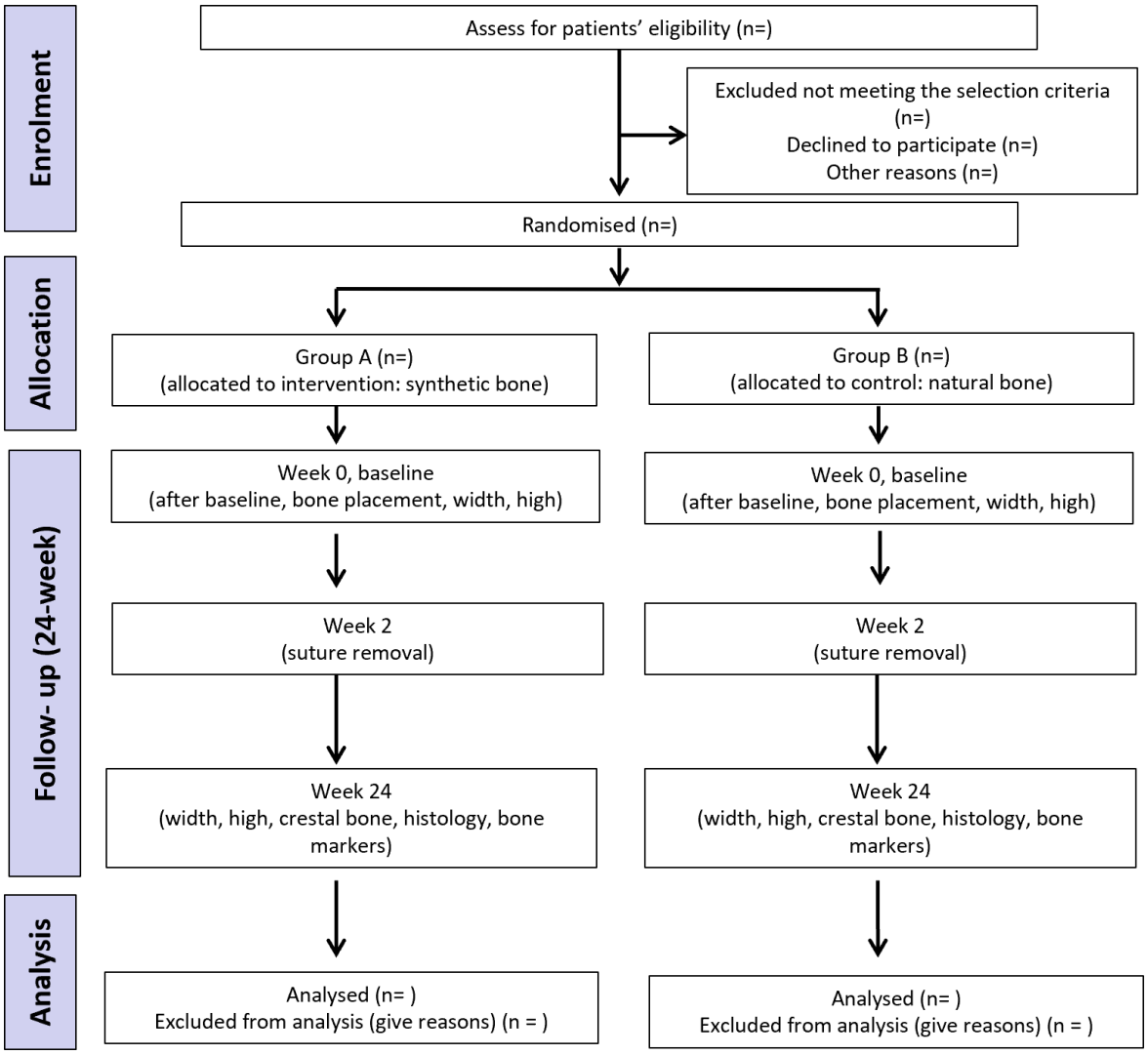
Laboratory processing of tissue samples for PCR

The procedure for processing the sample in the laboratory will be similar to that used in a study by Li et al. (25) Frozen tissue samples will be thawed in RNAlater®-ICE and lysed for RNA extraction using Qiagen RNeasy kit following the manufacturer's instructions. Following treatment with DNase-1 reagent and reverse-transcription of RNA samples using the SuperScript® VILOTM cDNA synthesis. SYBR green-based qPCR assays will be performed to analyse osteogenic gene expression, including Runx2, alkaline phosphatase, osteopontin and osteocalcin. The relative transcript levels of genes of interest will be analysed using the comparative CT (ÄÄCT) method. The range of relative transcript levels of the genes of interest will be presented as bar graphs, and statistical analysis will be performed at the level of ÄÄCT.

Table 1 Schedule of enrolment, intervention and assessments

	Study period				
	Enrolment	**Allocation**	**Post-allocation**		**Close-out**
Timepoint (weeks)	**-W0**	**W0**	**W0**	**W2**	**W24**
Enrolment					
Eligibility screen for patients	x				
Informed consent from patients	x				
List of other procedures	x				
Randomisation of patients		x			
Interventions					
Synthetic bone			x		
Natural bone			x		
Suture removal				x	
Assessments (Outcomes)					
Width			x		x
Height			x		x
Crestal bone					x
Histology					x
Bone Markers					x

Figure 1 CONSORT study flowchart



Criteria for discontinuing or modifying allocated interventions

- Participant withdraws consent
- The trial is discontinued
- Participant has an adverse reaction

The reasons for discontinuation will be documented (see Standard Operating Procedures).

Strategies for monitoring and improving protocol adherence

There are some measures that clinicians could adopt to improve patients' adherence to the research protocol, such as telephone calls, text reminders, and social support to educate patients. In addition, clinicians will have to create a welcoming, non-judgmental and accepting environment; educate patients about their role as research participants; establish a routine while maintaining flexibility; provide incentives for participation, parking spaces, and videoconferences.

Outcomes

Primary outcome

The primary outcome is the difference between the two low substitution rate bone substitutes, natural (Bio-Oss®) and synthetic (Bonalive®), in preserving the alveolar ridge dimensions assessed by changes in the width (expressed in millimetres) from augmentation (baseline) to 24-week post augmentation. Assessment will be done using intra-oral scanners (this does not involve radiographs) or impressions and clinical photographs.

Secondary outcomes

- Changes in height (expressed in millimetres) from augmentation (baseline) to 24-week post augmentation. Assessment will be done using the periapical radiographs taken pre-extraction and at 24-week follow-up and intra-oral scanners (this does not involve radiographs) or impressions and clinical photographs.
- Changes in the vertical crestal bone level (expressed in millimetres) assessed with periapical radiographs taken pre-extraction and 24-week post augmentation. The technique used for this assessment will be the same as that used by Solakoglu et al.(26) to evaluate changes in the alveolar bone crest in similar research.
- Presence of bone around the alveolar ridge assessed using a biopsy, histology (qualitative assessment of bone infiltration) and PCR to determine the concentration (in nanograms) of gene expression for bone markers (including osteopontin, osteocalcin) at 24-week post augmentation.

Sample size

According to Charles et al. (27) four factors are required to calculate the sample size: significance level, power, difference between groups and standard deviation. However, considering the limited availability of information in this area (only one study was identified conducted by Rignon-Bret et al.(28)

The research team, supported by the advice provided by statisticians, deiced to conduct a pilot study. Even though a pilot study does not require sample size calculation using the aforementioned four factors,(29) 15 to 20 participants per group are required to ensure the scientific validity of the pilot study results (30) . More recently, further information has been provided for the validity of the results of pilot RCT indicating a wider range of participants (31). Therefore, we used a pragmatic approach informed by the literature and we will recruit a total of 34 participants with 17 per group. The results of the pilot study will generate the four factors to calculate the sample size for the full-scale RCT.

Recruitment

Recruitment will be focused on private dental clinics in the Midlands, Southeast England and Northwest England

Clinicians: Experienced Implant surgeons at centres approved by lead researchers. Approval will be based on appropriate clinical setup, clinician experience and patient volume.

Patients: Patients who meet the inclusion criteria will be invited to take part in the study. They will consent to using either of the bone substitute materials and be randomly allocated to a group.

Recruitment will start as soon as ethics approval has been granted.

The recruitment strategy will increase potential participants' awareness of the health problem being studied and its potential impact on their health.

Clinician-patient ratio

The patients will be spread across 2-4 surgeries to ensure we meet the target sample size in a few months.

Randomisation, sequence generation, allocation and blinding

The randomisation, sequence generation and allocation concealment will be performed using the sealed envelope online system (https://www.sealedenvelope.com/) (32) . The randomisation will be 1:1. The patient will be the unit of randomisation and intervention.

All patients receiving the bone substitutes will be blinded to the treatment in our study. However, patients will know which bone substitute they have received after their final review at 24-week follow-up.

Data collection

Clinicians will collect data at baseline and at 24-week follow-up using a paper template. Then data will be transferred into Excel and SPSS and prepared for the data analysis. All paper records, except consent forms, will be destroyed at patients' last visit. Secure and irreversible destruction processes will include shredding and disposal of records using the University's confidential waste service.

Data management

The procedure will be conducted using the data management template provided by UKRI. Data will be managed following the procedure used in previous studies (26,28). Input data will

be saved and stored on a password-protected system. Only individuals authorised by the CI will be allowed to access the data. Paper data, such as Patients' informed consent will be kept in a locked cabinet by the clinicians.

Statistical methods

The primary analysis will be the intention-to-treat (ITT), including all randomised participants in the assigned group, regardless of their adherence to the protocol or their withdrawal.

Missing data will be assessed and treated using multiple imputations if missing at random (MAR).

To check for normality, each variable will be analysed using the Shapiro-Wilk test.

Primary outcome

The primary outcome is the difference between the two bone substitutes, natural (Bio-Oss®) and synthetic (Bonalive®), in preserving the alveolar ridge dimensions assessed by changes in the width of the alveolar ridge (expressed in millimetres) from augmentation (baseline) to 24-week post augmentation. It is expected to be a continuous variable, therefore, the comparison between groups at 24 weeks will be performed using a linear regression model (ANCOVA), including the baseline measurements as the covariates. The recent literature suggests using of ANCOVA as the statistical method of choice for the analysis of intervention effect and adjustment for baseline variables for three reasons: efficiency, precision and power (33).

Secondary outcomes

- The changes in the height of the alveolar ridge (expressed in millimetres) from grafting (baseline) to 24-week follow-up (post grafting) assessed using either intra-oral scanners or impressions and clinical photographs. It is expected to be a continuous variable; therefore, the comparison between groups at week 24 will be performed using the unpaired t-test for a normally distributed variable or the Mann-Whitney U test for non-normally distributed.
- The changes in vertical crestal bone levels between the groups (expressed in millimetres) will be compared using an unpaired t-test or Mann-Whitney U test according to the variable distribution. This will be assessed using radiographs pre-extraction and at 24-week follow-up.
- Presence of bone will be assessed using biopsy used for histology (qualitative assessment of bone infiltration) and PCR to determine the concentration (in nanograms) of gene expression of the bone markers (including osteopontin and osteocalcin).

- The comparison of the concentration of gene expression between the groups at 24-week will be performed using the unpaired t-test for a normally distributed variable or the Mann-Whitney U test for non-normally distributed

Data monitoring

This trial is designed to minimise the risk, as demonstrated in the previous trials by Rignon-Bret et al. (28)and Solakoglu et al. (26). Therefore, no formal committee has been organised, and no interim analysis of the impact of the intervention has been planned.

Risk and safety issues

The risk of allergy, infection and lack of bone integration will be monitored by the clinicians during the study keeping in contact with their patients.

Harm

We do not envisage any specific harm from these products, which are approved and in clinical use already, other than risks of potential complications from surgical procedure. This will be minimised through careful and appropriate case selection (healthy ASA Class I and II), surgical procedures with standard precautions for infection control and pre- and post-operative care. Additionally, the principal investigator enquired the Medical and Healthcare Products Regulatory Agency (MHRA) regarding the use of these bone substitutes in the study. The MHRA replied that "*as you are using the medical devices on human subjects **within** its CE marked intended use, as per the manufacturers IFU and the intention of the study is not to generate data to change or extend the indications of the CE marked device, you do not need to notify MHRA of this study"* (Appendix 8).

Auditing

No audit has been planned at this time.

Research ethics approval

Ethics approval will be sought from the Health Ethics Review Panel at the University of Central Lancashire (UCLan)

Protocol amendments

We are not expecting to make any changes to the eligibility criteria, outcomes or analyses during our study

Consent, invitation and confidentiality

All documentation related to information and consent for clinicians and patients has been enclosed in the protocol and approved by the UCLan ethics committees. The procedures followed for consent and confidentiality are described in the following paragraphs.

Informed consent

Clinicians participating in the study will recruit their patients and obtain consent from them.

Patients' recruitment and informed consent

After assessing patients' eligibility for the study, clinicians will provide an information letter and consent form to each patient, who will get a week to consider their participation. The clinicians will submit all signed consent forms to the PI so that they can be stored in the PI's office in a locked cabinet located within the School of Dentistry.

The baseline information will be transferred onto an Excel spreadsheet and stored electronically in a password protected UCLan computer. Anyone who is deemed unsuitable to participate will be offered a copy of their record and the electronic record will be destroyed in line with the University's confidential procedure.

Declaration of interest

None

Dissemination policy

The dissemination of the study will begin immediately with the publication of the protocol. The results of this trial will be presented at national and international conferences. They will be submitted as scientific manuscripts to peer-reviewed journals. The trial results aim to inform patients, policymakers, and all other stakeholders that might benefit from the results.

The results of the trial will be disseminated to service users and their families via media, to healthcare professionals via professional training and meetings, and researchers via conferences and publications. The publications generated by this study may be used as training materials for dentists with an interest in implantology.

Ancillary post-trial care

We are not envisaging the need for the provision of post-trial care. Nevertheless, all participants will be provided with an emergency contact number to reach the study

investigators so that they can receive the necessary support when they have any questions or problems.

Patient and Public Involvement (PPI)

The research protocol was developed during the COVID Omicron wave, February-March 2022, thus it was impossible to reach the patients and members of the public and get their input into the protocol.

Discussion

Following the extraction of teeth their supporting alveolar bone undergoes remodelling, which can result in the loss of height and width of the bony ridge; this is most marked in the aesthetic zone (anterior aspect of the upper jaw, the maxilla) (34,35).

To minimise this loss of ridge volume, the ridge preservation technique has been developed, which involves the insertion of bone substitute material to act as a space maintainer and scaffold for new bone growth (36–38)

The commonly used materials include bovine bone, human-donated bone and synthetic materials (6,38). There is a growing demand for non-animal products and the current synthetic materials have a high substitution rate which defeats the purpose of maintaining the space over periods longer than 4 to 5 months(10,12) . Bioactive glass (BAG) is a synthetic material with a low substitution rate, and antibacterial properties and is widely used by orthopaedic and ENT surgeons, but not yet by dental implant surgeons (13,14). This study aims to investigate whether the BAG material can be as effective or more effective as the bovine bone material as a bone substitute in implant dentistry. The importance of this study lies in its potential to facilitate a good ridge preservation and prevent more complicated augmentation procedures due to the loss of ridge volume in cases such as those listed below:

1. Aesthetic zone when immediate or early (4-8 weeks) placement is not possible (39)
2. Posterior maxilla – roots close to the floor of the sinus (40)
3. Posterior mandible – roots close to inferior dental canal/ mental foramen
4. Pontic area for aesthetics of the bridge
5. Extraction of the tooth adjacent to the implant
6. Large socket e.g., a molar with septal bone loss leaving a wide defect

In addition to the ridge preservation, benefits of BAG include reducing the risk of more complex, expensive, and risky augmentation procedures. The use of BAG can benefit patients undergoing sinus augmentation procedures, as well as the treatment of peri-implantitis (infection and bone loss around dental implants) due to its osteoconductive, osteostimulative, and antibacterial properties. .

Conclusion

Ridge preservation is a well-documented technique for maintaining the bone volume of the alveolar ridges of the jaws. This study will assess the effectiveness of the use of the synthetic material BAG which presents no cross-infection risk and has documented antibacterial properties and the desired low substitution rate. The results of this pilot study will provide the essential information to design a full-scale RCT and apply for funding too. Furthermore, the results of the pilot could be considered as the basis of further research into BAG use for general augmentation and in the treatment of peri-implantitis.

Protocol version

2.0

Funding

Not funded

Acknowledgements

In preparation of this protocol, Dr Miland Joshi acted as a critical friend in the development of the statistical analysis plan, so the research team would like to acknowledge this support. The research team is thankful to Dr Izabella Penier for editing the manuscript.

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Appendix

Appendix 1 ASA physical status classification system

ASA PS Classification	Definition	Adult Examples, Including, but not Limited to:
ASA I	A normal healthy patient	Healthy, non-smoking, no or minimal alcohol use
ASA II	A patient with mild systemic disease	Mild diseases only without substantive functional limitations. Current smoker, social alcohol drinker, pregnancy, obesity (30<BMI<40), well-controlled DM/HTN, mild lung disease
ASA III	A patient with severe systemic disease	Substantive functional limitations; One or more moderate to severe diseases. Poorly controlled DM or HTN, COPD, morbid obesity (BMI ≥40), active hepatitis, alcohol dependence or abuse, implanted pacemaker, moderate reduction of ejection fraction, ESRD undergoing regularly scheduled dialysis, history (>3 months) of MI, CVA, TIA, or CAD/stents.
ASA IV	A patient with severe systemic disease that is a constant threat to life	Recent (<3 months) MI, CVA, TIA or CAD/stents, ongoing cardiac ischemia or severe valve dysfunction, severe reduction of ejection fraction, shock, sepsis, DIC, ARD or ESRD not undergoing regularly scheduled dialysis
ASA V	A moribund patient who is not expected to survive without the operation	Ruptured abdominal/thoracic aneurysm, massive trauma, intracranial bleed with mass effect, ischemic bowel in the face of significant cardiac pathology or multiple organ/system dysfunction
ASA VI	A declared brain-dead patient whose organs are being removed for donor purposes	

American Society of Anaesthesiologists (2022) *ASA Physical Status Classification*

Appendix 2 Instructions for the use of Bonalive

Please note the following when using the product.
Please also read the instructions for use.

Quick guide
Bonalive® granules

Non-hardening synthetic and bacterial growth inhibiting bone cavity filler used for filling, replacement and reconstruction of bone defects.

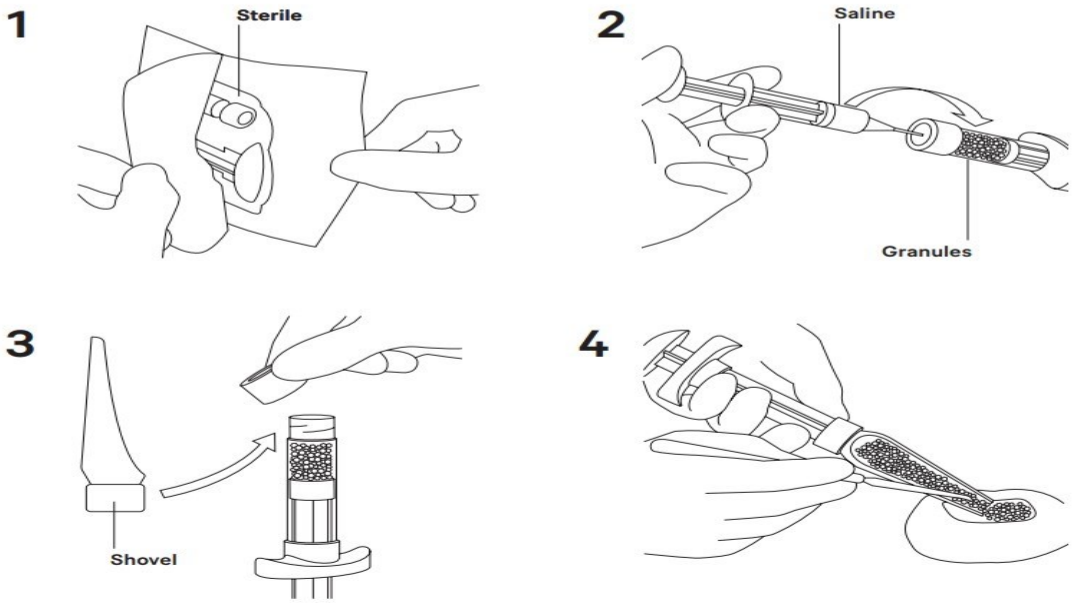
The granules are moistened prior to implantation. The procedure facilitates implantation of granules and activates bioactive reactions. Moisten the granules by injecting sterile saline solution through the cap membrane.

Unscrew the cap and screw the shovel tightly onto the applicator body, in order to prevent spilling of the granules keep the opening facing upwards. Implant the moistened granules from the shovel into the defect with a sterile instrument. Can be mixed with autologous bone graft in a sterile cup and subsequently implanted with a sterile instrument.

A thorough mechanical debridement of the defect must be carried out and the surface of the bone must be refreshed.

The application should have sufficient bony walls and the cavity should be properly filled with the granules as the material does not shrink or expand. Misplaced granules must be removed from outside the defect. Avoid direct skin contact by closing the defect well.

Antibiotics and other implantation related therapies should be used in accordance with routine hospital practices.



bonalive

Based on instruction for use version 91301/10
Version 91310e/2

Appendix 3 Instructions for the use of Bio-Oss®

- Cut the Geistlich Bio-Oss® Collagen block to the appropriate size.
- Apply it dry or moisten with saline solution.
- When moistened, mould to the desired shape.
- Place in defect site with forceps.

- Avoid excessive compression.

Appendix 4 Packaging instruction

P650	PACKING INSTRUCTION	P650
This packing instruction applies to UN No. 3373.		

1. The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during carriage, including transhipment between cargo transport units and between cargo transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings shall be constructed and closed to prevent any loss of contents that might be caused under normal conditions of carriage by vibration or by changes in temperature, humidity, or pressure.
2. The packaging shall consist of at least three components:
    0. a primary receptacle.
    1. a secondary packaging; and
    2. an outer packaging
3. Primary receptacles shall be packed in secondary packagings in such a way that, under normal conditions of carriage, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings shall be secured in outer packagings with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.
4. For carriage, the mark illustrated below shall be displayed on the external surface of the outer packaging on a background of a contrasting colour and shall be clearly visible and legible. The mark shall be in the form of a square set at an angle of 45° (diamond-shaped) with minimum dimensions of 50 mm by 50 mm; the width of the line shall be at least 2 mm and the letters and numbers shall be at least 6 mm high. The proper shipping name "BIOLOGICAL SUBSTANCE, CATEGORY B" in letters at least 6 mm high shall be marked on the outer packaging adjacent to the diamond-shaped mark.

5.

of which either the secondary or the outer packaging shall be rigid.

(5) At least one surface of the outer packaging shall have a minimum dimension of 100 mm × 100 mm.

he completed package shall be capable of successfully passing the drop test in 6.3.5.3 as specified in 6.3.5.2 at a height of 1.2 m. Following the appropriate drop sequence, there shall be no leakage from the primary receptacle(s) which shall remain protected by absorbent material, when required, in the secondary packaging.

P650	PACKING INSTRUCTION *(cont'd)*	P650

1. For liquid substances:
   0. The primary receptacle(s) shall be leakproof;
   1. The secondary packaging shall be leakproof;
   2. If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;
   3. Absorbent material shall be placed between the primary receptacle(s) and the secondary packaging. The absorbent material shall be in quantity sufficient to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
   4. The primary receptacle or the secondary packaging shall be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar).
1. For solid substances:
   0. The primary receptacle(s) shall be siftproof;
   1. The secondary packaging shall be siftproof;
   2. If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;
   3. If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during carriage then a packaging suitable for liquids, including absorbent materials, shall be used.
1. Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen:
   0. When dry ice or liquid nitrogen is used as a coolant, the requirements of 5.5.3 shall apply. When used, ice shall be placed outside the secondary packagings or in the outer packaging or an overpack. Interior supports shall be provided to secure the secondary packagings in the original position. If ice is used, the outside packaging or overpack shall be leakproof.
   1. The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures which could result if refrigeration were lost.

(10) When packages are placed in an overpack, the package marks required by this packing instruction shall either be clearly visible or be reproduced on the outside of the overpack.

(11) Infectious substances assigned to UN No. 3373 which are packed and packages which are marked in accordance with this packing instruction are not subject to any other requirement in ADR.

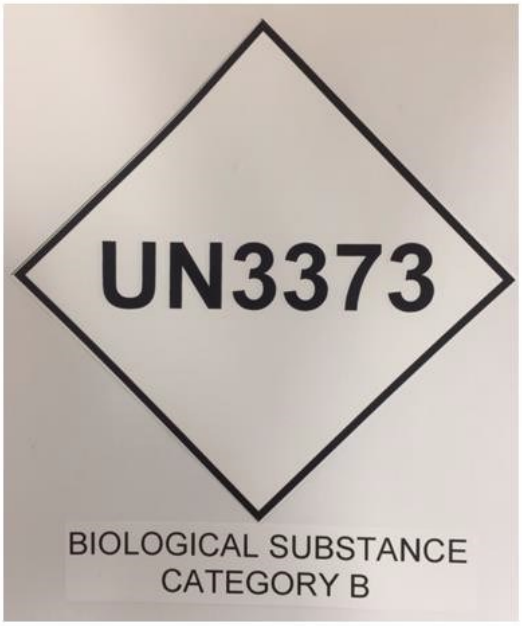
(12) Clear instructions on filling and closing such packages shall be provided by packaging manufacturers and subsequent distributors to the consignor or to the person who prepares the package (e.g. patient) to enable the package to be correctly prepared for carriage.

(13) Other dangerous goods shall not be packed in the same packaging as Class 6.2 infectious substances unless they are necessary for maintaining the viability, stabilising or preventing degradation or neutralising the hazards of the infectious substances. A quantity of 30 ml or less of dangerous goods included in Classes 3, 8 or 9 may be packed in each primary receptacle containing infectious substances. When these small quantities of dangerous goods are packed with infectious substances in accordance with this packing instruction no other requirements of ADR need be met.

(14) If any substance has leaked and has been spilled in a cargo transport unit, it may not be reused until after it has been thoroughly cleaned and, if necessary, disinfected or decontaminated. Any other goods and articles carried in the same vehicle or container shall be examined for possible contamination.

Appendix 5 Biological substance category label

UN3373 Packaging Label Example:



Appendix 6: HTA License for ICHTB

HTA
Human Tissue Authority

Licensing Number	**12275**
Licence Holder	**Imperial College Healthcare NHS Trust**
Licensed Premises	**Imperial College London** **South Kensington Campus** **Exhibition Road** **London** **SW7 2AZ**
Designated Individual	**Professor Iain Alexander McNeish**

This licence is granted under Section 16 (2) (e) (ii) of the Human Tissue Act 2004.

This licence authorises the storage of relevant material which has come from a human body for use for the following scheduled purposes:

- Determining the cause of death
- Establishing after a person's death the efficacy of any drug or other treatment administered to him
- Obtaining scientific or medical information about a living or deceased person which may be relevant to any other person (including a future person)
- Public display
- Research in connection with disorders, or the functioning, of the human body
- Clinical audit
- Education or training relating to human health
- Performance assessment
- Public health monitoring
- Quality assurance

The licensed activity should be carried on only at the licensed premises specified above, and under the supervision of the Designated Individual.

This licence is subject to the conditions set out in the Annexes accompanying this licence as may be subsequently varied pursuant to an application under paragraph 8 of Schedule 3 to the Human Tissue Act 2004.

This licence is valid from the date specified below and will remain in force until revoked.

.....
Bill Horne
Chair

.....
Nicolette Harrison
Director of Regulatory Delivery

Valid From **01 August 2019**

An independent statutory regulator sponsored by Department of Health

Appendix 7 Dry ice label

UN1845 Dry Ice Packaging Label Example:
Example:

Appendix 8 Email from MHRA

From: Hashem, Lina <Lina.Hashem@mhra.gov.uk>
Sent: Friday, July 2, 2021 5:13:43 PM
To: Fadi N Barrak <School of Medicine> <FNBarrak@uclan.ac.uk>
Subject: RE: MHRA approval enquiry Reference: E/2021/0937

Dear Fadi

Reference: E/2021/0937

Apologies for the delay.

You have stated you wish to conduct a study to compare two bone substitute materials for use in implant dentistry.

You have stated that both products are CE marked and will be used within their stated intended use. We note that the results will be published in a dental journal to improve knowledge and provide evidence for this potentially antibacterial bone substitute material with a view to improve patient care especially with the treatment of peri-implantitis.

As you are using the medical devices on human subjects **within** its CE marked intended use, as per the manufacturers IFU and the intention of the study is not to generate data to change or extend the indications of the CE marked device, you do not need to notify MHRA of this study.

Please note that whilst we are willing to give any help and advice we can, any views given by us on the interpretation of the legislation represent our best judgement at the time, based on the information available. Such views are not meant to be a definitive statement of law, which may only be given by the Courts. Accordingly we would always advise you to seek the views of your own professional advisors.

Kind Regards,

Lina

Lina Hashem
Regulatory Affairs Specialist
Devices Regulatory Group