Lampiran I
Nomor : 0267/E5/AK.04/2022
Tanggal : 28 April 2022

PENERIMA PENDANAAN PENELITIAN PROGRAM KOMPETITIF NASIONAL DAN PENUGASAN DI PERGURUAN TINGGI TAHUN ANGGARAN 2022 TAHAP PERTAMA

NOMOR	PTN/ LLDIKTI	INSTITUSI	NIDN	NAMA	JUDUL	SKEMA
1	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0109029701	ANGGUN SYAFITRI	EFEKTIVITAS KOMBINASI EKSTRAK DAUN BENALU DUKU (Dendrophtoe Pentandra (L.)Miq) DAN LENDIR SIPUT (Achatina fulica) SEBAGAI REPAIRING SKIN DALAM FORMULASI SEDIAAN SERUM	PDP
2	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0101108103	BERLIANA R BR NAIPOSPOS	The Speech Act Used By English Lecturer and Students in Blended Learning During New Normal Life: Pragmatic Linguistic Analysis	PDP
3	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0112056501	HAPOSAN SIAHAAN	Uji Efek Tonikum Ekstrak Etanol Daun Senggani (Melastoma Malabathricum L.) Terhadap Mencit Jantan (Mus Musculus)	PDP
4	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0130109202	HARIATI	HUBUNGAN SELF EFFICACY, SELF ESTEEM, DAN LOKUS KENDALI TERHADAP KEPATUHAN LANSIA MELAKSANAKAN PROTOKOL KESEHATAN PENCEGAHAN COVID-19	PDP
5	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0121108902	HERVIANI SARI	Formulasi dan uji daya terima substitusi garam dapur (NaCl) dengan ekstrak daun basil (Ocimum Basilicum L.) terfortifikasi Iodium terhadap penurunan resiko hipertensi pada lansia	PDP
6	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0101039202	REISY TANE	PENGARUH VICARIOUS LEARNING VIDEO RESPONSIVE FEEDING TERHADAP POLA MAKAN DAN KENAIKAN BERAT BADAN ANAK USIA 6-24 BULAN DI KABUPATEN DELI SERDANG	PDP
7	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0130118003	RENTAWATI PURBA	Perbedaan respon psikologis perawat IGD RSU Sembiring dalam menghadapi virus covid 19 dan varian baru virus corona di era adaptasi kebiasaan baru.	PDP
8	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0125028203	ROSTIODERTINA GIRSANG	ANALISIS FAKTOR YANG BERHUBUNGAN DENGAN SELF EFFICACY PENDERITA TB PARU MENJALANI PENGOBATAN DI PUSKESMAS DELITUA	PDP
9	LLDIKTI I	Institut Kesehatan Deli Husada Deli Tua	0110058702	SOFIA RAHMI	Efektivitas Anti-Aging Sediaan Serbuk Masker Kombinasi Ampas Tahu dan Kolang-Kaling (Arenga pinnata)	PDP
10	LLDIKTI I	Institut Kesehatan Helvetia	0106099301	MUHAMMAD ANDRY	Uji Aktivitas Antibakteri Streptococcus Mutans Serta Formulasi Sediaan Pasta Gigi Ekstrak Etanol Buah Okra Hijau (Abelmoschus Esculentus) dan Tulang Ikan Tuna (Thunnini)	PDP

NOMOR	PTN/ LLDIKTI	INSTITUSI	NIDN	NAMA	JUDUL	SKEMA
3988	Negeri PTNBH	Universitas Airlangga	8865610016	CITA ROSITA SIGIT PRAKOESWA	Pengaruh pemberian Epigallocatechin-3-gallate (EGCG) terhadap ekspresi dari transforming growth factor-β2 (TGF-β2), matrix metalloproteinase-1 (MMP-1), collagen typeI dan level trans epidermal water loss (TEWL) pada pasien photoaging	PPS-PDD
3989	Negeri PTNBH	Universitas Airlangga	0013107202	DENI KUSUMAWARDANI	AGLOMERASI EKONOMI, POLUSI, DAN KRIMINALITAS DI INDONESIA: ANALISIS DATA MIKRO	PPS-PTM
3990	Negeri PTNBH	Universitas Airlangga	0013107202	DENI KUSUMAWARDANI	MODAL SOSIAL DAN PERILAKU PEDULI LINGKUNGAN HIDUP DI INDONESIA: ANALISIS DATA MIKRO	PPS-PTM
3991	Negeri PTNBH	Universitas Airlangga	0003057602	DIAH INDRIANI	Imputasi Data Missing dengan Pendekatan k-Neirest Neighbor untuk Pendugaan Model Regresi Spasial (Studi Kasus Kejadian Stunting di Kabupaten Blitar)	PPS-PTM
3992	Negeri PTNBH	Universitas Airlangga	0020086105	DIAN AGUSTIA	Hubungan visualisasi Integrated Sustainability Reporting (ISR) dan pengambilan keputusan investasi dalam mendukung green economy	PPS-PDD
3993	Negeri PTNBH	Universitas Airlangga	0020086105	DIAN AGUSTIA	CSR Intensity, CSR Assurance & Firm Value: The role on sustainable development goals	PPS-PTM
3994	Negeri PTNBH	Universitas Airlangga	0020087102	DIAN AGUSTIN WAHJUNINGRUM	OPTIMASI PRODUK BIOCERAMIC BCP-Sr-Ag SCAFFOLD DENGAN KOMBINASI DENTAL PULP STEM CELLS (DPSCs) - CONDITIONED MEDIUM (CM) SEBAGAI BONE GRAFT	PTKN
3995	Negeri PTNBH	Universitas Airlangga	0007126806	DYAH WULAN SARI	AKSES KELEMBAGAAN DAN KEMISKINAN RUMAH TANGGA NELAYAN PERIKANAN TANGKAP DI INDONESIA	PPS-PDD
3996	Negeri PTNBH	Universitas Airlangga	0022096905	EDI DWI RIYANTO	Strategi Adaptasi Masyarajat Hindu-Bali di Kota Surabaya melalui Implementasi Tri Hita Karana	PPS-PTM
3997	Negeri PTNBH	Universitas Airlangga	0007107801	ELIDA ZAIRINA	Faktor - Faktor yang Memengaruhi Polifarmasi pada Pasien Geriatri di Rumah Sakit	PPS-PDD
3998	Negeri PTNBH	Universitas Airlangga	0009086102	ELLY MUNADZIROH	Biokomposite Bovine Amniotic Membrane Hidroksiapatit sebagai Kandidat Material Untuk Preservasi Soket setelah Pencabutan Gigi	PPS-PDD
3999	Negeri PTNBH	Universitas Airlangga	0022027102	ENDANG RETNO SURJANINGRUM	Dukungan Sosial pada Penyintas Kekerasan dalam Rumah Tangga: Perspektif Masyarakat Pedesaan	PPS-PTM
4000	Negeri PTNBH	Universitas Airlangga	0022027102	ENDANG RETNO SURJANINGRUM	Basic Symptom yang Menentukan Status Clinical High Risk Terhadap Psikosis pada Individu yang Mencari Bantuan	PPS-PDD
4001	Negeri PTNBH	Universitas Airlangga	0012026603	ERNIE MADURATNA SETIAWATIE	Produksi dan Aplikasi bovine amniotic membran - hyaluronic acid untuk pengobatan regeneratif dibidang kedokteran gigi	PTKN

UNIVERSITAS AIRLANGGA
LEMBAGA PENELITIAN DAN PENGABDIAN MASYARAKAT
Kampus C Mulyorejo Surabaya 60115 - Telp. (031) 5995247 Fax. (0315923584)
laman: http://lppm.unair.ac.id; e-mail: penelitian@lppm.unair.ac.id, pengmas@lppm.unair.ac.id

KONTRAK PENELITIAN
SKEMA PENELITIAN TERAPAN KOMPETITIF NASIONAL (PTKN)
TAHUN ANGGARAN 2022
NOMOR: 846/UN3.15/PT/2022

Pada hari ini **Kamis** tanggal **Dua Belas** bulan **Mei** tahun **Dua Ribu Dua Puluh Dua,** kami yang bertanda tangan di bawah ini:

1. **Dr. Gadis Meinar Sari, dr., M.Kes.** : Ketua Lembaga Penelitian dan Pengabdian Masyarakat Universitas Airlangga, dalam hal ini bertindak untuk dan atas nama Universitas Airlangga, yang berkedudukan di Kampus C Universitas Airlangga, Mulyorejo - Surabaya untuk selanjutnya disebut **PIHAK KESATU;**
2. **Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).** : Dosen Fakultas Kedokteran Gigi Universitas Airlangga, dalam hal ini bertindak sebagai pengusul dan Ketua Pelaksana Penelitian Tahun Anggaran 2022 untuk selanjutnya disebut **PIHAK KEDUA**.

PIHAK KESATU dan **PIHAK KEDUA**, secara bersama-sama selanjutnya disebut **PARA PIHAK,** bersepakat mengikatkan diri dalam suatu Kontrak Penelitian Skema Penelitian Terapan Kompetitif Nasional (PTKN) Tahun Anggaran 2022 dengan ketentuan dan syarat-syarat sebagai berikut:

PASAL 1
RUANG LINGKUP

PIHAK KESATU memberikan pendanaan kepada **PIHAK KEDUA** dan **PIHAK KEDUA** menerima pendanaan tersebut dari **PIHAK KESATU,** untuk melaksanakan dan menyelesaikan Penelitian Terapan Kompetitif Nasional Tahun Anggaran 2022 dengan judul:

OPTIMASI PRODUK BIOCERAMIC BCP-Sr-Ag SCAFFOLD DENGAN KOMBINASI DENTAL PULP STEM CELLS (DPSCs) - CONDITIONED MEDIUM (CM) SEBAGAI BONE GRAFT

PASAL 2
SUMBER DANA

PIHAK KESATU memberikan pendanaan Kontrak penelitian yang bersumber pada DIPA Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi Tahun Anggaran 2022, Nomor SP DIPA- Nomor SP DIPA-023.17.1.690523/2022 revisi ke-02 Tanggal 22 April 2022.

PASAL 3
NILAI KONTRAK

(1) **PIHAK KESATU** memberikan pendanaan Kontrak Penelitian kepada **PIHAK KEDUA** dengan nilai kontrak sebesar **Rp 250.000.000,00 (Dua Ratus Lima Puluh Juta Rupiah)** yang di dalam nilai kontrak tersebut sudah termasuk seluruh biaya pajak sesuai peraturan perundang-undangan.

(2) Pendanaan pelaksanaan program penelitian dengan nilai kontrak sebagaimana dimaksud pada ayat (1) dibayarkan kepada **PIHAK KEDUA** sebagai berikut:

Nama Pemilik Rekening	: **Ibu DIAN AGUSTIN WAHJUNINGRUM**
Nomor Rekening	: **0706754560**
Nama Bank	: **Bank Negara Indonesia (BNI)**

(3) **PIHAK KESATU** tidak bertanggungjawab atas keterlambatan dan/atau tidak terbayarnya sejumlah dana, yang disebabkan oleh kesalahan **PIHAK KEDUA** dalam menyampaikan informasi sebagaimana dimaksud pada ayat (2).

PASAL 4
NILAI DAN TAHAPAN PEMBAYARAN

(1) Dana pelaksanaan penelitian sebagaimana nilai kontrak yang dimaksud dalam Pasal 3 ayat (1) dibayarkan oleh **PIHAK KESATU** kepada **PIHAK KEDUA** secara bertahap melalui Bank Negara Indonesia (BNI) Cabang Unair kepada rekening ketua peneliti melalui mekanisme transfer, dengan ketentuan sebagai berikut:
   a. Pembayaran tahap pertama sebesar **Rp 175.000.000,00 (Seratus Tujuh Puluh Lima Juta Rupiah)** setelah **PIHAK KEDUA** mengirimkan dokumen kontrak yang telah ditandatangani;
   b. Pembayaran tahap pertama sebagaimana dimaksud pada huruf a, akan dibayarkan dengan ketentuan apabila revisi proposal penelitian dan surat pernyataan kesanggupan pelaksanaan penelitian telah diunggah ke laman yang ditentukan oleh **PIHAK KESATU**;
   c. Pembayaran tahap kedua sebesar **Rp 75.000.000,00 (Tujuh Puluh Lima Juta Rupiah),** dibayarkan setelah pelaksana peneliti mengunggah Surat Pernyataan Tanggung Jawab Belanja (SPTB) ke laman yang ditentukan oleh **PIHAK KESATU** paling lambat tanggal **16 Agustus 2022;** dan
   d. Apabila pembayaran tahap pertama sebagaimana dimaksud pada huruf a cair setelah tanggal 9 Agustus 2022, pelaksana penelitian mengunggah Surat Pernyataan Tanggung Jawab Belanja (SPTB) ke laman yang ditentukan oleh **PIHAK KESATU** paling lambat 2 (dua) minggu setelah dana cair.

(2) Khusus penelitian lanjutan, keberlanjutan pendanaan untuk tahun anggaran berikutnya diberikan berdasarkan hasil penilaian atas capaian penelitian tahun sebelumnya yang dilakukan oleh Komite Penilaian Keluaran Penelitian dan/atau *Reviewer* Keluaran Penelitian.

(3) **PIHAK KEDUA** harus menyampaikan surat pernyataan telah menyelesaikan seluruh pekerjaan yang dibuktikan dengan pengunggahan pada laman yang ditentukan oleh **PIHAK KESATU** paling lambat tanggal **20 November 2022**, dengan melampirkan dokumen sebagai berikut:
   a. Surat Pernyataan Tanggung Jawab Belanja (SPTB); dan
   b. Laporan Akhir Pelaksanaan Pekerjaan.

(4) Khusus untuk dana pembayaran 30% yang baru cair setelah tanggal 13 November 2022, **PIHAK KEDUA** mengunggah dokumen sebagaimana dimaksud pada ayat (3) paling lambat 2 (dua) minggu setelah dana dicairkan.

PASAL 5
JANGKA WAKTU PENYELESAIAN

Jangka waktu pelaksanaan penelitian dimulai sejak tanggal **16 Maret hingga 20 November 2022.**

PASAL 6
KEWAJIBAN DAN HAK

(5) **PIHAK KESATU** mempunyai kewajiban:
a. memberikan pendanaan penelitian kepada **PIHAK KEDUA**;
b. melakukan pemantauan dan evaluasi;
c. melakukan penilaian luaran penelitian; dan
d. melakukan validasi luaran tambahan.

(6) **PIHAK KEDUA** mempunyai kewajiban melaksanakan **Kontrak Penelitian** dan mengunggah ke laman yang ditentukan oleh **PIHAK KESATU** atas dokumen sebagai berikut:
1. Revisi Proposal Penelitian;
2. Surat Pernyataan Kesanggupan Pelaksanaan Penelitian;
3. Catatan Harian Pelaksanaan Penelitian;
4. Laporan Kemajuan Pelaksanaan Penelitian;
5. Surat Pernyataan Tanggung Jawab Belanja (SPTB) atas dana penelitian yang telah ditetapkan;
6. Laporan Akhir Penelitian; dan
7. Luaran Penelitian.

(7) **PIHAK KESATU** mempunyai hak menerima dokumen hasil unggahan di laman yang ditentukan **PIHAK KESATU** sebagai berikut:
1. Revisi Proposal Penelitian;
2. Surat Pernyataan Kesanggupan Pelaksanaan Penelitian;
3. Catatan Harian Pelaksanaan Penelitian;
4. Laporan Kemajuan Pelaksanaan Penelitian;
5. Surat Pernyataan Tanggung Jawab Belanja (SPTB) atas dana penelitian yang telah ditetapkan;
6. Laporan Akhir Penelitian; dan
7. Luaran Penelitian.

(8) **PIHAK KEDUA** mempunyai hak mendapatkan dana penelitian dari **PIHAK KESATU.**

PASAL 7
PENGGANTIAN KEANGGOTAAN

(1) Perubahan terhadap susunan tim pelaksana penelitian dan substansi penelitian dapat dibenarkan apabila telah mendapat persetujuan dari Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi

(2) Apabila Ketua Tim Pelaksana Penelitian tidak dapat menyelesaikan penelitian atau mengundurkan diri, maka **PIHAK KESATU** berhak menunjuk pengganti Ketua Tim Pelaksana Penelitian yang merupakan salah satu anggota tim dengan

mempertimbangkan masukan dari anggota tim dan setelah mendapat persetujuan tertulis dari Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi.

(3) Dalam hal tidak adanya pengganti Ketua Tim Pelaksana Penelitian sesuai dengan syarat dan ketentuan dalam panduan penelitian, maka penelitian dibatalkan dan dana dikembalikan ke Kas Negara.

PASAL 8
LUARAN PENELITIAN

(1) **PIHAK KEDUA** berkewajiban untuk mencapai target luaran wajib penelitian berupa **Dokumen Pendaftaran Paten Proses: Terbit Nomor Pendaftaran Paten,** dan mengunggahnya ke laman yang ditentukan oleh **PIHAK KESATU**.

(2) **PIHAK KEDUA** diharapkan mencapai luaran tambahan penelitian berupa **Artikel pada Conference/ Seminar Internasional di Pengindeks Bereputasi: Terbit dalam Prosiding; Artikel ilmiah pada Jurnal Internasional Bereputasi 200 Terbaik (Q1: Accepted,** dan mengunggahnya ke laman yang ditentukan oleh **PIHAK KESATU**.

(3) **PIHAK KEDUA** berkewajiban untuk mencantumkan sumber pendanaan pada setiap publikasi atau bentuk apapun yang berkaitan dengan hasil penelitian ini yakni **Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi.**

PASAL 9
MONITORING DAN EVALUASI

PIHAK KESATU dalam rangka koordinasi, pengawasan, dan pemantauan, akan melakukan Monitoring dan Evaluasi (Monev) terhadap kemajuan pelaksanaan penelitian Tahun Anggaran 2022.

PASAL 10
PAJAK

Ketentuan pengenaan pajak pertambahan nilai dan/atau pajak penghasilan dalam rangka pelaksanaan kegiatan penelitian ini wajib dilaksanakan oleh **PIHAK KEDUA** sesuai dengan ketentuan peraturan perundang-undangan di bidang perpajakan.

PASAL 11
KEKAYAAN INTELEKTUAL

(1) Hak Kekayaan Intelektual yang dihasilkan dari pelaksanaan penelitian diatur dan dikelola sesuai dengan peraturan dan perundang-undangan.

(2) Setiap publikasi, makalah, dan/atau ekspos dalam bentuk apapun yang berkaitan dengan hasil penelitian ini wajib mencantumkan **Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi,** sebagai pemberi dana penelitian.

(3) Pencantuman nama pihak pemberi dana sebagaimana dimaksud pada ayat (2), paling sedikit mencantumkan nama **Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi.**

(4) Hasil penelitian berupa peralatan dari kegiatan ini adalah milik negara dan dapat dihibahkan kepada institusi/ lembaga melalui Berita Acara Serah Terima (BAST) untuk keberlanjutan pengembangan penelitian, dicatat secara tertib dan akuntabel dalam inventaris barang PTNBH sesuai dengan peraturan Perundang-undangan.

PASAL 12
INTEGRITAS AKADEMIK

(1) Pelaksana penelitian wajib menjunjung tinggi integritas akademik yaitu komitmen dalam bentuk perbuatan yang berdasarkan pada nilai kejujuran, kredibilitas, kewajaran, kehormatan, dan tanggung jawab dalam kegiatan penelitian yang dilaksanakan.

(2) Penelitian dilakukan sesuai dengan kerangka etika, hukum, dan profesionalitas, serta kewajiban sesuai dengan peraturan yang berlaku.

(3) Penelitian dilakukan dengan menjunjung tinggi standar ketelitian dan integritas tertinggi dalam semua aspek penelitian.

PASAL 13
KEADAAN KAHAR/ MEMAKSA

(1) **PARA PIHAK** dibebaskan dari tanggung jawab atas keterlambatan atau kegagalan dalam memenuhi kewajiban yang dimaksud dalam **Kontrak Penelitian** disebabkan atau diakibatkan oleh peristiwa atau kejadian di luar kekuasaan **PARA PIHAK** yang dapat digolongkan sebagai keadaan memaksa (*force majeure*).

(2) Peristiwa atau kejadian yang dapat digolongkan keadaan memaksa (*force majeure*) sebagaimana dimaksud pada ayat (1) meliputi bencana alam, wabah penyakit, kebakaran, perang, blokade, peledakan, sabotase, revolusi, pemberontakan, huru-hara, serta adanya tindakan pemerintah dalam bidang ekonomi dan moneter yang secara nyata berpengaruh terhadap pelaksanaan **Kontrak Penelitian** ini.

(3) Apabila terjadi keadaan memaksa (*force majeure*) maka pihak yang mengalami wajib memberitahukan kepada pihak lainnya secara tertulis, selambat-lambatnya dalam waktu 7 (tujuh) hari kerja sejak terjadinya keadaan memaksa (*force majeure*), disertai dengan bukti-bukti yang sah dari pihak yang berwajib, dan **PARA PIHAK** dengan itikad baik akan segera membicarakan penyelesaiannya.

PASAL 14
PENYELESAIAN PERSELISIHAN

(1) Apabila terjadi perselisihan atau perbedaan penafsiran terkait Kontrak Penelitian ini, **PARA PIHAK** sepakat untuk menyelesaikannya secara musyawarah dan mufakat.

(2) Dalam hal tidak tercapai penyelesaian secara musyawarah dan mufakat sebagaimana dimaksud pada ayat (1) maka penyelesaian dilakukan melalui proses hukum yang berlaku dengan memilih domisili hukum di Pengadilan Negeri Surabaya.

PASAL 15
AMANDEMEN KONTRAK

Apabila terdapat hal lain yang belum diatur atau terjadi perubahan dalam **Kontrak Penelitian** ini, maka akan dilakukan amandemen Kontrak Penelitian.

PASAL 16
SANKSI

(1) Apabila sampai dengan batas waktu yang telah ditetapkan untuk melaksanakan **Kontrak Penelitian** telah berakhir, **PIHAK KEDUA** tidak melaksanakan kewajiban sebagaimana dimaksud dalam Pasal 6 ayat (2), maka **PIHAK KEDUA** dikenai sanksi administratif sesuai dengan ketentuan peraturan perundang-undangan.

(2) Apabila di kemudian hari terbukti bahwa judul proposal yang diajukan pada program penelitian sebagaimana dimaksud dalam Pasal 1 ditemukan adanya duplikasi dan/atau ditemukan adanya ketidakjujuran/itikad buruk yang tidak sesuai dengan kaidah ilmiah, maka kegiatan penelitian tersebut dinyatakan batal dan **PIHAK KEDUA** dikenai sanksi administratif.

(3) Sanksi administratif sebagaimana dimaksud pada ayat (1) dan (2) dapat berupa penghentian pembayaran dan/atau Ketua Tim Pelaksana Penelitian tidak dapat mengajukan proposal penelitian dalam kurun waktu 2 (dua) tahun berturut-turut.

PASAL 17
PENUTUP

Kontrak Penelitian ini dibuat dan ditandatangani oleh **PARA PIHAK** pada hari dan tanggal tersebut di atas, dibuat dalam rangkap 2 (dua) asli bermeterai cukup yang biayanya dibebankan kepada **PIHAK KEDUA,** yang masing – masing mempunyai kekuatan hukum yang sama.

PIHAK KESATU	**PIHAK KEDUA**
UNIVERSITAS AIRLANGGA LEMBAGA PENELITIAN DAN PENGABDIAN MASYARAKAT	SEPULUH RIBU RUPIAH 10000 METERAI TEMPEL 53523AJX897452061
Dr. Gadis Meinar Sari, dr., M.Kes.	**Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).**
NIDN 0004056612	**NIDN 0020087102**

No. 846/SPTJM/Tahap-I/2022

SURAT PERNYATAAN TANGGUNGJAWAB MUTLAK

Hibah Program Penelitian DRTPM Tahun Anggaran 2022

Yang bertanda tangan di bawah ini

1.	N a m a	:	Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).
2.	N I D N	:	0020087102
3.	Jabatan	:	Ketua Peneliti
4.	Fak/Lembaga	:	Universitas Airlangga
5.	SK Rektor	:	1004/UN3/2022
6.	Sumber Dana	:	Kemendikbud-Ristek RI Tahun Anggaran 2022
7.	Nilai Kontrak	:	Rp. **250.000.000**- (Dua Ratus Lima Puluh Juta Rupiah)
8.	Tahap I	:	Rp. **175.000.000**- (Seratus Tujuh Puluh Lima Juta Rupiah)
9.	Skema	:	Penelitian Terapan Kompetitif Nasional
10.	Judul	:	Optimasi Produk Bioceramic Bcp-Sr-Ag Scaffold Dengan Kombinasi Dental Pulp Stem Cells (Dpscs) - Conditioned Medium (Cm) Sebagai Bone Graft

Menyatakan dengan sesungguhnya bahwa :

1. Bertanggungjawab secara mutlak sesuai dengan RAB dalam pembelanjaan dana penelitian dan bersedia menyimpan bukti-buktinya sesuai dengan standar biaya yang berlaku;
2. Berkewajiban mengembalikan sisa dana yang tidak dibelanjakan ke Kas Negara/Universitas Airlangga;
3. Berkewajiban memungut dan menyetor pajak-pajak sesuai ketentuan yang berlaku;
4. Berkewajiban membuat Berita Acara Serah Terima ke Universitas Airlangga apabila melakukan transaksi pembelian barang modal.

Demikian Surat Pernyataan ini saya buat dengan sebenarnya.

Surabaya, 20 Juni 2022
Ketua Peneliti,

Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).
NIDN. 0020087102

No. 846/SPTJM/Tahap-II/2022

SURAT PERNYATAAN TANGGUNGJAWAB MUTLAK

Hibah Program Penelitian DRTPM Tahun Anggaran 2022

Yang bertanda tangan di bawah ini

1.	N a m a	:	Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).
2.	N I D N	:	0020087102
3.	Jabatan	:	Ketua Peneliti
4.	Fak/Lembaga	:	Universitas Airlangga
5.	SK Rektor	:	1004/UN3/2022
6.	Sumber Dana	:	Kemendikbud-Ristek RI Tahun Anggaran 2022
7.	Nilai Kontrak	:	Rp. **250.000.000**- (Dua Ratus Lima Puluh Juta Rupiah)
8.	Tahap II	:	Rp. **75.000.000**- (Tujuh Puluh Lima Juta Rupiah)
9.	Skema	:	Penelitian Terapan Kompetitif Nasional
10.	Judul	:	Optimasi Produk Bioceramic Bcp-Sr-Ag Scaffold Dengan Kombinasi Dental Pulp Stem Cells (Dpscs) - Conditioned Medium (Cm) Sebagai Bone Graft

Menyatakan dengan sesungguhnya bahwa :

1. Bertanggungjawab secara mutlak sesuai dengan RAB dalam pembelanjaan dana penelitian dan bersedia menyimpan bukti-buktinya sesuai dengan standar biaya yang berlaku;
2. Berkewajiban mengembalikan sisa dana yang tidak dibelanjakan ke Kas Negara/Universitas Airlangga;
3. Berkewajiban memungut dan menyetor pajak-pajak sesuai ketentuan yang berlaku;
4. Berkewajiban membuat Berita Acara Serah Terima ke Universitas Airlangga apabila melakukan transaksi pembelian barang modal.

Demikian Surat Pernyataan ini saya buat dengan sebenarnya.

Surabaya, 20 Juni 2022
Ketua Peneliti,

Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).
NIDN. 0020087102

No. 846/SPJ/Tahap-I/2022

SURAT PERTANGGUNGJAWABAN DANA PENELITIAN

UNIVERSITAS AIRLANGGA

Sesuai dengan : 1. U.U. Nomor 17 Tahun 2003 tentang Keuangan Negara;
2. U.U. Nomor 1 Tahun 2004 tentang Perbendaharaan Negara;
3. U.U. Nomor 20 Tahun 2003 tentang Sistem Pendidikan Nasional;
4. U.U. Nomor 12 Tahun 2012 tentang Pendidikan Tinggi;
5. P.P. Nomor 57 Tahun 1954 tentang Pendirian Universitas Airlangga di Surabaya sebagaimana telah diubah dengan Peraturan Pemerintah nomor : 3 Tahun 1955 tentang pengubahan Peraturan Pemerintah Nomor : 1954;
6. P.P. Nomor 37 Tahun 2009 tentang Dosen;
7. P.P. Nomor 30 Tahun 2014 tentang Statuta Universitas Airlangga;
8. Peraturan Wali Amanat Universitas Airlangga.

Unit Kerja : 2 0 2 0 0 Lembaga Penelitian Dan Pengabdian Masyarakat Universitas Airlangga

Kode Kegiatan :

Kode Rekening :

Telah Terima : Rektor Universitas Airlangga

Terbilang Rp. : Seratus Tujuh Puluh Lima Juta Rupiah

Untuk Pembayaran : Penelitian Terapan Kompetitif Nasional

Judul : Optimasi Produk Bioceramic Bcp-Sr-Ag Scaffold Dengan Kombinasi Dental Pulp Stem Cells (Dpscs) - Conditioned Medium (Cm) Sebagai Bone Graft

Sumber Dana : Kemendikbud-Ristek RI Tahun Anggaran 2022

Termin : I

Ketua Peneliti : Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).

Jumlah : **Rp. 175.000.000**

Surabaya, 20 Juni 2022

Lunas Dibayar Bendahara

Ketua Peneliti

Wishnu Okky Pranadi Tirta
NIP. 199210232018013101

Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).
NIDN. 0020087102

Mengetahui / Menyetujui
Atasan Langsung Bendahara

Gadis Meinar Sari
NIP. 196605041996032001

No: 846/SPJ/Tahap-II/2022

SURAT PERTANGGUNGJAWABAN DANA PENELITIAN

UNIVERSITAS AIRLANGGA

Sesuai dengan : 1. U.U. Nomor 17 Tahun 2003 tentang Keuangan Negara;
2. U.U. Nomor 1 Tahun 2004 tentang Perbendaharaan Negara;
3. U.U. Nomor 20 Tahun 2003 tentang Sistem Pendidikan Nasional;
4. U.U. Nomor 12 Tahun 2012 tentang Pendidikan Tinggi;
5. P.P. Nomor 57 Tahun 1954 tentang Pendirian Universitas Airlangga di Surabaya sebagaimana telah diubah dengan Peraturan Pemerintah nomor : 3 Tahun 1955 tentang pengubahan Peraturan Pemerintah Nomor : 1954;
6. P.P. Nomor 37 Tahun 2009 tentang Dosen;
7. P.P. Nomor 30 Tahun 2014 tentang Statuta Universitas Airlangga;
8. Peraturan Wali Amanat Universitas Airlangga.

Unit Kerja : 20200 Lembaga Penelitian Dan Pengabdian Masyarakat Universitas Airlangga

Kode Kegiatan :

Kode Rekening :

Telah Terima : Rektor Universitas Airlangga

Terbilang Rp. : Tujuh Puluh Lima Juta Rupiah

Untuk Pembayaran : Penelitian Terapan Kompetitif Nasional

Judul : Optimasi Produk Bioceramic Bcp-Sr-Ag Scaffold Dengan Kombinasi Dental Pulp Stem Cells (Dpscs) - Conditioned Medium (Cm) Sebagai Bone Graft

Sumber Dana : Kemendikbud-Ristek RI Tahun Anggaran 2022

Termin : II

Ketua Peneliti : Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).

Jumlah : **Rp. 75.000.000**

Surabaya, 20 Juni 2022

Lunas Dibayar Bendahara

Ketua Peneliti

Wishnu Okky Pranadi Tirta
NIP. 199210232018013101

Dr. Dian Agustin Wahjuningrum, drg., Sp.KG(K).
NIDN. 0020087102

Mengetahui / Menyetujui
Atasan Langsung Bendahara

Gadis Meinar Sari
NIP. 196605041996032001

Isian Substansi Proposal

PENELITIAN TERAPAN KOMPETITIF NASIONAL (PTKN)

Petunjuk:Pengusul hanya diperkenankan mengisi di tempat yang telah disediakan sesuai dengan petunjuk pengisian dan tidak diperkenankan melakukan modifikasi template atau penghapusan di setiap bagian.

Tuliskan judul usulan penelitian

JUDUL USULAN

OPTIMASI PRODUK *BIOCERAMIC* BCP-Sr-Ag SCAFFOLD DENGAN KOMBINASI *DENTAL PULP STEM CELLS* (DPSCs) - *CONDITIONED MEDIUM* (CM) SEBAGAI *BONE GRAFT*

Ringkasan penelitian tidak lebih dari 500 kata yang berisi latar belakang penelitian, tujuan dan tahapan metode penelitian, luaran yang ditargetkan, serta uraian TKT penelitian yang diusulkan.

RINGKASAN

Bone grafting merupakan prosedur transplantasi jaringan tulang untuk memperbaiki dan membentuk kembali tulang yang telah rusak. Kerusakan tulang dapat disebabkan oleh fraktur dan proses infeksi. Menurut data statistik lebih dari dua juta prosedur *grafting* tulang dilakukan per tahunnya[1,2], menjadikan transplantasi tulang sebagai transplantasi jaringan terbesar kedua yang dilakukan proses transplantasi setelah transfusi darah. Hal ini menjadi permasalahan kesehatan di Indonesia karena tingginya angka ketergantungan terhadap produk kesehatan luar negeri sehingga meningkatkan *health service cost*. *Biphasic calcium phosphate* (BCP) merupakan *bioactive ceramic* material komersial yang dapat digunakan sebagai *bone graft*. Peningkatan karakteristik BCP dapat dilakukan dengan *dopping* Strontium dan Silver. Strontium akan meningkatkan kemampuan *mechanical properties* BCP dan silver akan meningkatkan kemampuan daya antibakteri. Saat ini telah dilakukan pembuatan *bioceramic* BCP-Sr-Ag pada skala mikro. Optimasi BCP-Sr-Ag perlu dilakukan untuk meningkatkan *osteogenic induction* dengan kombinasi *Dental pulp stem cells conditioned medium* (DPSCs-CM). Inovasi BCP-Sr-Ag kombinasi *Dental pulp stem cells conditioned medium* menjadi penelitian unggulan fakultas kedokteran gigi Universitas Airlangga yang diharapkan dapat menjadi solusi permasalahan kebutuhan produk *bone graft* di Indonesia. BCP-Sr-Ag kombinasi DPSCs-CM memiliki keunggulan dibanding *bone graft* komersial lainnya yaitu sekaligus memiliki kemampuan daya antibakteri. Hal ini meningkatkan keberhasilan penyembuhan tulang yang rusak karena kerusakan tulang biasanya disertai dengan infeksi. Saat ini telah dilakukan Uji *mechanical properties* menggunakan XRD dan SEM untuk memastikan *mechanical properties* BCP-Sr-Ag pada skala mikro. Optimasi dilakukan untuk meningkatkan *osteogenic induction* BCP-Sr-Ag yang di uji menggunakan *flowcytometry* dan IHC. Melalui aktifitas Penelitian Terapan Kompetitif Nasional diharapkan dapat mengakselerasi peningkatan TKT BCP-Sr-Ag kombinasi *Dental pulp stem cells conditioned medium* menjadi TKT 6 dengan hasil *intellectual properties* paten dan publikasi ilmiah lainnya hingga dihasilkan produk *bioceramic bone graft* BCP-Sr-Ag yang dikombinasikan dengan *Dental pulp stem cells conditioned medium*.

Kata kunci maksimal 5 kata

KATA KUNCI

Bone graft; BCP-Sr-Ag; DPSCs-CM

Latar belakang penelitian tidak lebih dari 500 kata yang berisi latar belakang dan permasalahan yang akan diteliti, tujuan khusus dan studi kelayakannya. Pada bagian ini perlu dijelaskan uraian tentang spesifikasi keterkaitan skema dengan bidang fokus atau renstra penelitian PT.

LATAR BELAKANG

Bone grafting merupakan prosedur transplantasi jaringan tulang untuk memperbaiki dan membentuk kembali tulang yang telah rusak. Kerusakan tulang dapat disebabkan oleh fraktur dan proses infeksi. Menurut data statistik lebih dari dua juta prosedur *grafting* tulang dilakukan per tahunnya[1,2], menjadikan transplantasi tulang sebagai transplantasi jaringan terbesar kedua yang dilakukan proses transplantasi setelah transfusi darah. Hal ini menjadi permasalahan karena *bone graft* yang tersedia di Indonesia merupakan produk import sehingga mengakibatkan tingginya biaya pelayanan kesehatan tulang dan ketergantungan produk import. *Bone graft* diperlukan untuk memperbaiki kerusakan tulang karena memiliki sifat osteogenesis, osteokonduksi, dan osteointegrasi. Bahan *bone graft* terdiri dari empat, yaitu: *autograft*, *allograft*, *xenograft*, dan *alloplastic materials*. *Bone graft autograft* merupakan *gold standard*[2,3]. Kekurangan *autograft* adalah bioavailabilitas yang terbatas serta adanya komplikasi post operasi[4]. Kekurangan *allograft* dan *xenograft* yaitu adanya resiko transmisi penyakit[2].

Saat ini penggunaan material *alloplastic* mulai banyak digunakan yaitu *Hydroxyapatite* (HA), β-*Tricalcium phosphate* (β-TCP), dan *Biphasic Calcium Phosphate* (BCP)[5,6]. HA adalah jenis *alloplastic materials* yang sering digunakan namun HA memiliki kekurangan solubilitas yang rendah. β-TCP memiliki struktur berporus sehingga mempercepat proses remodeling tulang, namun mudah larut jika terkena *body fluid*[7,8]. Penggabungan HA dan β-TCP menghasilkan BCP. BCP merupakan material *bioceramic composite* yang memiliki kelebihan *biological properties* dapat diatur melalui porositas yang dipengaruhi oleh rasio HA/β-TCP[9].

Karakteristik BCP dapat ditingkatkan dengan penambahan material *ionic* yaitu melalui doping Strontium (Sr) dan Silver (Ag). Strontium akan meningkatkan kemampuan *mechanical properties* BCP; meningkatkan aktivitas osteoblast; inhibitor osteoklas dan sitokin pro-inflamasi. Dosis optimum ion Sr^{2+} pada konsentrasi 3 mol %. Silver akan meningkatkan kemampuan daya antibakteri dengan dosis optimum pada konsentrasi 1 mol %[10-13]. Optimasi *osteogenic* dapat dilakukan dengan kombinasi *Dental pulp stem cells conditioned medium* (DPSCs-CM). DPSCs-CM akan meningkatkan kemampuan osteoinduksi *biomaterial composite* BCP. Inovasi *bioceramic* BCP doping Sr^{2+}+Ag^{+}+DPSCs-CM akan meningkatkan proses regenerasi tulang melalui penulangan jalur endokondral dengan peningkatan faktor osteogenik yaitu RUNX2, ALP, OPN maupun OCN dan faktor angiogenik yaitu VEGFR-2 dan BMP-2.

Inovasi BCP-Sr-Ag kombinasi DPSCs-CM merupakan penelitian unggulan fakultas kedokteran gigi sesuai dengan Renstra Universitas Airlangga dengan fokus Kesehatan dibidang pengembangan *stem cells* diharapkan dapat menjadi solusi permasalahan kebutuhan produk *bone graft* di Indonesia. Kelebihan BCP-Sr-Ag kombinasi DPSCs-CM selain kemampuan osteogenesisnya juga memiliki kemampuan daya antibakteri. Saat ini telah dilakukan pembuatan BCP dengan ratio 60:40 menghasilkan *bone graft* dengan porositas yang ideal. Porositas akan memfasilitasi *stem cells* dan *growth factor* untuk bersinergi meningkatkan osteogenesis. *Doping* Sr dan Ag dilakukan untuk meningkatkan karakteristik BCP pada skala mikro telah diuji menggunakan XRD dan SEM. Optimasi dilakukan untuk meningkatkan *osteogenic induction* BCP-Sr-Ag yang di uji menggunakan *flowcytometry* dan IHC. Melalui aktifitas Penelitian Terapan Kompetitif Nasional diharapkan dapat mengakselerasi peningkatan TKT BCP-Sr-Ag menjadi TKT 6 dengan hasil *intellectual properties* paten dan publikasi ilmiah lainnya hingga

dihasilkan produk *bone graft* BCP-Sr-Ag dengan kombinasi dengan *Dental pulp stem cells conditioned medium*.

Urgency penelitian

1. Tingginya angka kebutuhan *bone graft* per tahun (2.2 miliar prosedur *bone grafting*) dan keterbatasan *autograft* sebagai material *gold standard* pada prosedur *bone grafting*
2. Tingginya angka ketergantungan produk Kesehatan import
3. Terbatasnya *bone graft* dengan kemampuan osteogenik dan angiogenik sekaligus memiliki kemampuan daya anti bakteri.

Tinjauan pustaka tidak lebih dari 1000 kata dengan mengemukakan *state of the art* dan peta jalan (*roadmap*) dalam bidang yang diteliti/teknologi yang dikembangkan. Penyajian peta jalan dapat berupa bagan dalam bentuk *image*. Sumber pustaka/referensi primer yang relevan dan dengan mengutamakan hasil penelitian pada jurnal ilmiah dan/atau paten yang terkini.

TINJAUAN PUSTAKA

Bone grafting merupakan prosedur transplantasi jaringan tulang untuk memperbaiki dan membentuk kembali tulang yang telah rusak. *Bone graft* dapat memperbaiki kerusakan tulang karena memiliki sifat osteogenesis, osteokonduksi, osteoinduksi, dan osteointegrasi.

Kerusakan tulang dapat terjadi karena trauma, infeksi bakteri, maupun kekurangan nutrisi, khususnya kalsium dan vitamin D sehingga diperlukan optimasi *bone graft* dengan kemampuan daya antibakteri.

Bone graft berdasarkan sumbernya dibagi menjadi 4 tipe, yaitu *autograft*, *xenograft*, *allograft*, dan *alloplastic materials*[2]. *Autograft* merupakan *bone graft* yang didapatkan dari individu yang sama dengan penerima, merupakan *gold standard* karena memiliki sifat osteoinduktif, osseointegrasi, osteogenesis, dan osteokonduktif[2,3]. Kekurangan *autograft* adalah bioavailabilitas yang terbatas sehingga diperlukan pengembangan biomaterial lain sebagai alternatif yaitu *alloplast* atau *alloimplant*. *Alloplast* terbentuk dari bahan sintetik golongan kalsium fosfat[4] yang terdiri dari: *hydroxyapatite* (HA), *Beta-tricalcium phosphate* (β-TCP), dan *Biphasic Calcium Phosphate* (BCP)[5,6].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) merupakan material yang sering digunakan sebagai *bone graft* namun kekurangan HA adalah solubilitas yang rendah (ratio Ca/P=1:1.67)[7,8]. β-TCP ($Ca_3(PO_4)_2$) memiliki struktur berporus sehingga mempercepat proses *remodeling* tulang. Kekurangan β-TCP adalah tingkat kelarutan dan terdegradasi dengan cepat jika terkena *body fluid* (rasio Ca/P=1:1.5)[7,8].

BCP merupakan gabungan dari HA dan β-TCP[14]. Kelebihan BCP memiliki *biological properties* yang dapat diatur dengan rasio HA/β-TCP[15]. Kelemahan BCP memiliki *mechanical strength* yang kurang; osteoinduktif yang masih rendah; dan struktur *intrinsic brittleness*.

Peningkatan karakteristik BCP dilakukan dengan penggabungan material lain menjadi *material bioceramic*[9]. Penggabungan material lain melalui *single layer doping* untuk memperbaiki karakteristik maupun *multi-layer* agar memiliki daya antibakteri sehingga dapat meningkatkan kemampuan osteogenesisnya. *Doping* dapat dilakukan melalui penyisipan partikel inorganik yang bersifat *ionic* menggunakan *cross-linked* sehingga tidak menghasilkan *large aggregate*.

Sr^{2+} (Strontium) merupakan material *ionic* yang dapat meningkatkan aktivitas *osteoblast* dan menurunkan aktivitas osteoklas dan meningkatkan osteogenik *differentiation*[16]. Dosis optimum

ion Sr^{2+} pada konsentrasi 3 mol % memiliki osteokonduktifitas yang lebih baik dengan menstimulasi aktifitas proliferasi osteogenik dan angiongenik[10].

Ag^{+} (Silver) merupakan material *ionic* yang dapat menghambat pertumbuhan bakteri dengan dosis ion Ag^{+} yang digunakan sebagi bahan *doping* yaitu pada konsentrasi 1 mol %[10-13].

Osteogenesis erat kaitannya dengan angiogenesis melalui jalur *endochondral ossification*. *Endochondral ossification* adalah penyembuhan tulang jalur indirek dimana terjadi pembentukan *soft callus, hard callus, woven*, dan *lamellar bone* yang terdiri dari *repair, reparative*, dan *remodeling*. Tahap awal repair diikuti dengan pembentukan hematoma dan sel neutrofil. Neutrofil merupakan attraktan makrofag (M1), melepaskan sitokin pro-inflamasi, dan merekrut *Mesenchymal Stem Cells* (MSCs) endogenous ke tempat fraktur. Sitokin pro-inflamatori merupakan sitokin penting untuk memulai cascade inflamasi dan proses penyembuhan fraktur[17,18].

Tahapan repair ditandai dengan disintesisnya RUNX2 yang merupakan faktor osteogenesis utama yang diikuti dengan sintesis ALP, OPN, dan OCN dan faktor angiogenik utama yang diikuti dengan sintesis VEGFR-2 dan BMP-2 dengan hasil akhir diferensiasi MSCs menjadi *osteoblast*. MSCs *endogenous* yang direkrut menuju ke tempat fraktur oleh neutrofil akan meningkatkan pembentukan tulang secara *in vivo* (osteokonduksi). Penambahan MSC *exogenous* akan meningkatkan pembentukan tulang melalui diferensiasi menjadi kondroblas dan *osteoblast* yang merupakan osifikasi secara endokondral[17,18]. Sampai saat ini pemanfaatan MSCs masih belum optimal karena prosedur *stem cell* memerlukan pelaksanaan-penyimpanan-penanganan yang *highly qualified*, sehingga harganya relatif mahal, dan sel yang telah diimplantasi tidak dapat bertahan lama[18-20].

Dental Pulp Stem Cells (DPSCs) merupakan salah satu jenis *Mesenchymal Stem Cells* (MSCs) yang dapat diisolasi dari gigi molar ketiga manusia[21,22]; sumber potensial osteogenik yang dapat berdiferensisasi menjadi *osteoblast*[22]; dapat digunakan bersama dengan bubuk HA/TCP untuk membentuk tulang lamelar pada permukaan HA/TCP[22]; dan merupakan stem cell *multipoten* yang dapat meregenerasi, proliferasi, dan diferensiasi menjadi sel jenis lain.

Perkembangan *stem cell* mengarah ke pemanfaatan *conditioned medium* (CM) yang dapat diproses dalam jumlah besar *(mass product)*; bertahan lama sehingga bersifat ekonomis dan *marketable*[23,24]. CM merupakan kultur stem cell pada medium dengan rentang level oksigen tertentu. Rentang level oksigen disebut *normoxia* bila konsentrasi 20-21%, dan *hypoxia* pada konsentrasi 1-13%[25,26]. Oksigen dapat meningkatkan toksisitas CM melalui pembentukan *Reactive Oxygen Species* (ROS)[27]. *Hypoxia* memodulasi potensi angiogenik melalui peningkatan *vasculoendothelial growth factor* (VEGF), *basic fibroblast growth factor* (bFGF), dan *Hepatocyte Growth Factor* (HGF) yang dihubungkan dengan aktivitas *Hypoxia Inducible Factor* (HIF) 1-2α[27].

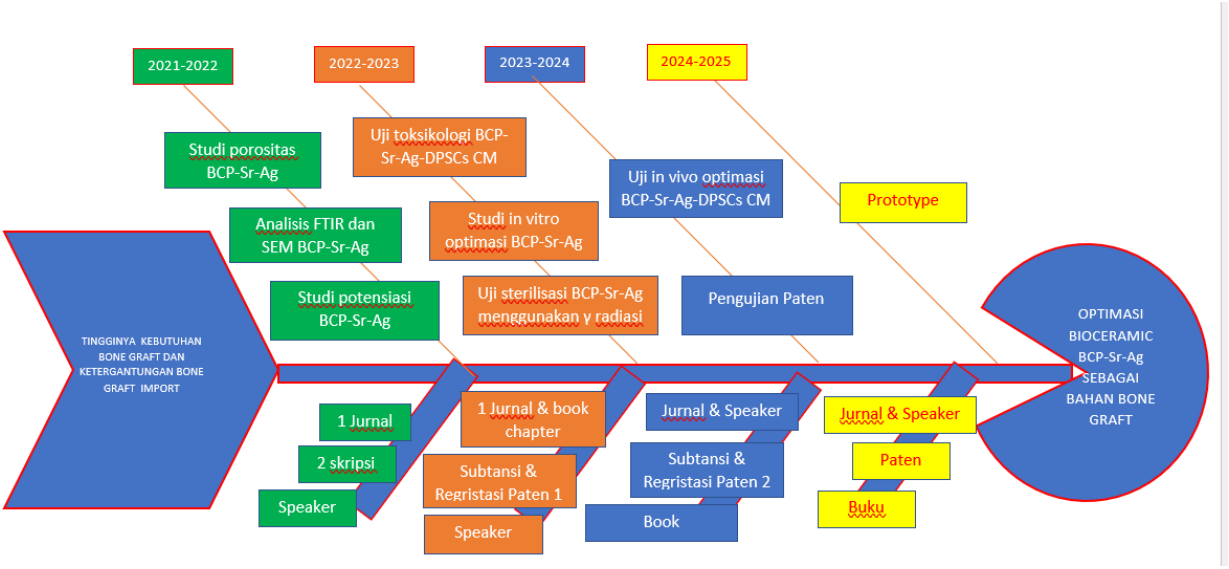
Penambahan CM akan meningkatkan *growth factor*, sitokin, dan molekul matriks ekstraseluler. *Growth Factor* dan sitokin DPSC-CM dapat berfungsi meningkatkan kemampuan osteoinduktif bila dikombinasikan pada *bio-composite* BCP-Sr^{2+}-Ag^{+}sehingga perlu dilakukan inovasi produk *bioceramic* BCP-Sr^{2+}-Ag^{+} *scaffold* yang dikombinasikan dengan DPSCs-CM sebagai *bone graft.*

State the art

Tingginya angka kebutuhan *bone graft* per tahun sebesar 2.2 milyar menempatkan tulang sebagai organ yang paling banyak dilakukan transplantasi kedua setelah darah. Tingginya angka kebutuhan *bone graft* tidak diimbangi dengan ketersediaan *bone graft* produk dalam negeri. *Bone graft* komersial produk import menyebabkan tingginya ketergantungan terhadap produk import.

Permasalahan ini perlu disikapi dengan produk inovatif *bone graft* karya anak bangsa Indomesia. BCP-Sr-Ag dengan kombinasi DPSCs-CM memiliki keunggulan dibanding *bone graft* kompetitif lainnya yaitu selain memiliki kemampuan *osteoinduction* yang dioptimasi dengan DPSCs-CM sekaligus memiliki kemampuan daya antibakteri. Pengendalian infeksi pasca pemasangan *bone graft* akan meningkatkan keberhasilan pembentukan tulang yang baru.

Roadmap penelitian.



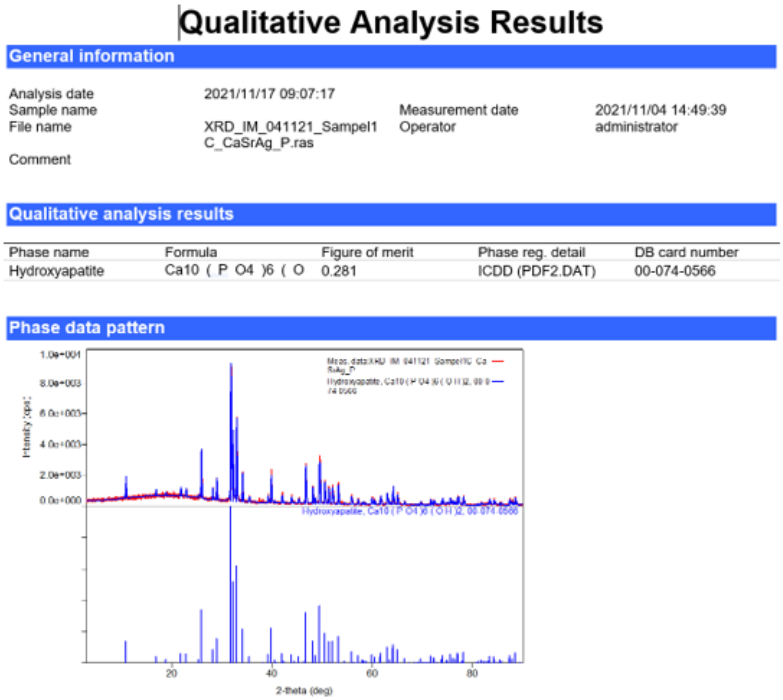
Metode atau cara untuk mencapai tujuan yang telah ditetapkan ditulis tidak melebihi 600 kata. Bagian ini dilengkapi dengan diagram alir penelitian yang akan dikerjakan selama waktu yang diusulkan. Bagan penelitian harus dibuat secara utuh dengan penahapan yang jelas, semua tahapan untuk mecapai luaran beserta indikator capaian yang ditargetkan. Pada bagian ini harus juga dijelaskan tugas masing-masing anggota pengusul sesuai tahapan penelitian yang diusulkan.

METODA

Jenis penelitian ini adalah penelitian *True Experimental Laboratoris* dimana menggunakan rancangan *posttest only group control design*. Penelitian ini akan dilakukan di Surabaya (*Dental Research Centre*, Fakultas Kedokteran Gigi Universitas Airlangga); *School of Materials and Mineral Resources Engineering*, Universiti Sains Malaysia-Malaysia; *Department Stem Cells* Universiti Putra Malaysia)-Malaysia dan PT Energi Sterila Higiena yang memiliki fasilitas *Electron beam machine and X-Ray*.

BCP-Sr-Ag pada skala mikro yang telah selesai dilakukan pada tahun 2021.

Hasil uji SEM juga telah selesai dilakukan.



Qualitative Analysis Results

General information

Analysis date	2021/11/17 09:07:17		
Sample name		Measurement date	2021/11/04 14:49:39
File name	XRD_IM_041121_Sampel1C_CaSrAg_P.ras	Operator	administrator
Comment			

Qualitative analysis results

Phase name	Formula	Figure of merit	Phase reg. detail	DB card number
Hydroxyapatite	Ca10 (P O4)6 (O	0.281	ICDD (PDF2.DAT)	00-074-0566

Phase data pattern

Hasil uji kristalisasi dan ukuran partikel.

Crystallite Size and Lattice Strain

General information

Analysis date	2021/11/17 09:07:10		
Sample name		Measurement date	2021/11/04 14:49:39
File name	XRD_IM_041121_Sampel1C_CaSrAg_P.ras	Operator	administrator
Comment			

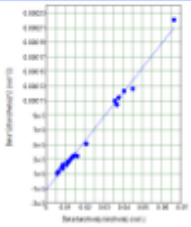
Qualitative analysis results

Phase name	Formula	Figure of merit	Phase reg. detail	DB card number
Hydroxyapatite	Ca10 (P O4)6 (O	0.281	ICDD (PDF2.DAT)	00-074-0566

Crystallite size and lattice strain

Phase name	Crystallite size(Å)	Strain(%)
Hydroxyapatite	480(10)	0.000000

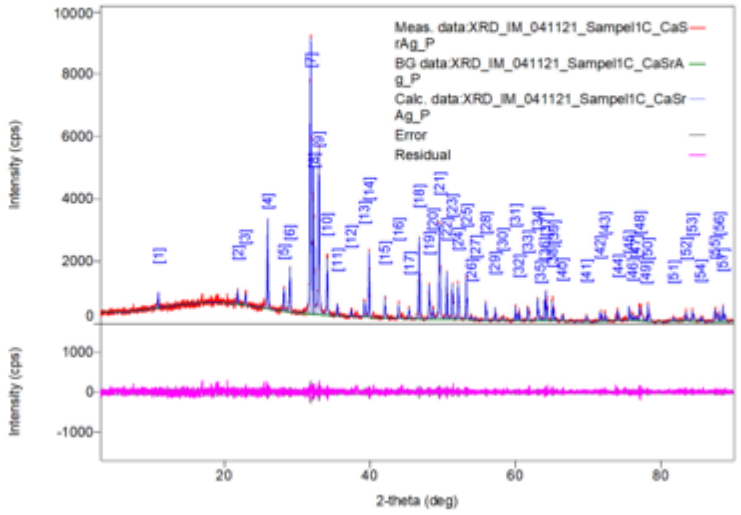
Equation
Y=0.00337X-0.0000134



Crystallinity

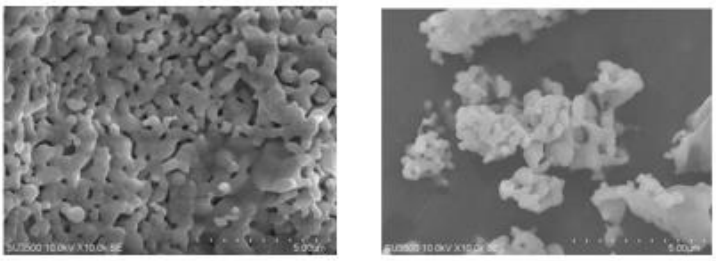
Data set name	Crystallinity(%)
XRD_IM_041121_Sampel1C_CaSrAg_P	83.5(5)

Measurement profile

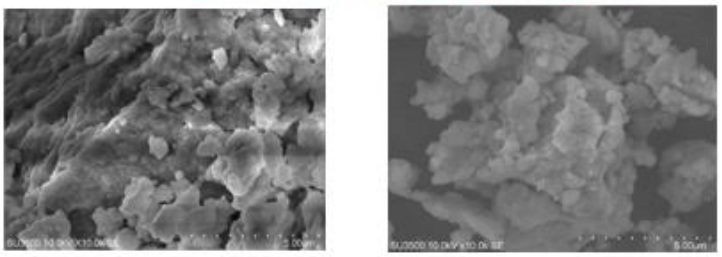


Hasil uji SEM EDX.

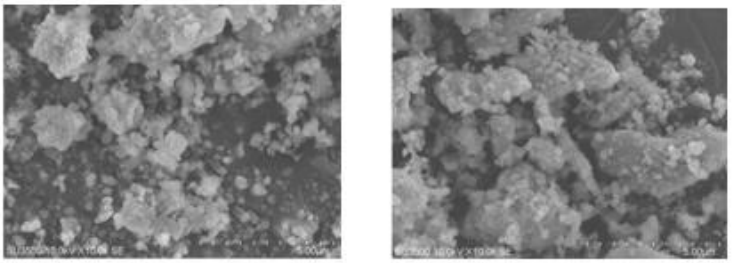
Hasil pengukuran SEM-EDS



Gambar 1. Morfologi HA setelah dikalsinasi (sampel 1)



Gambar 2. Morfologi HA setelah dikalsinasi + GTA (sampel 3)

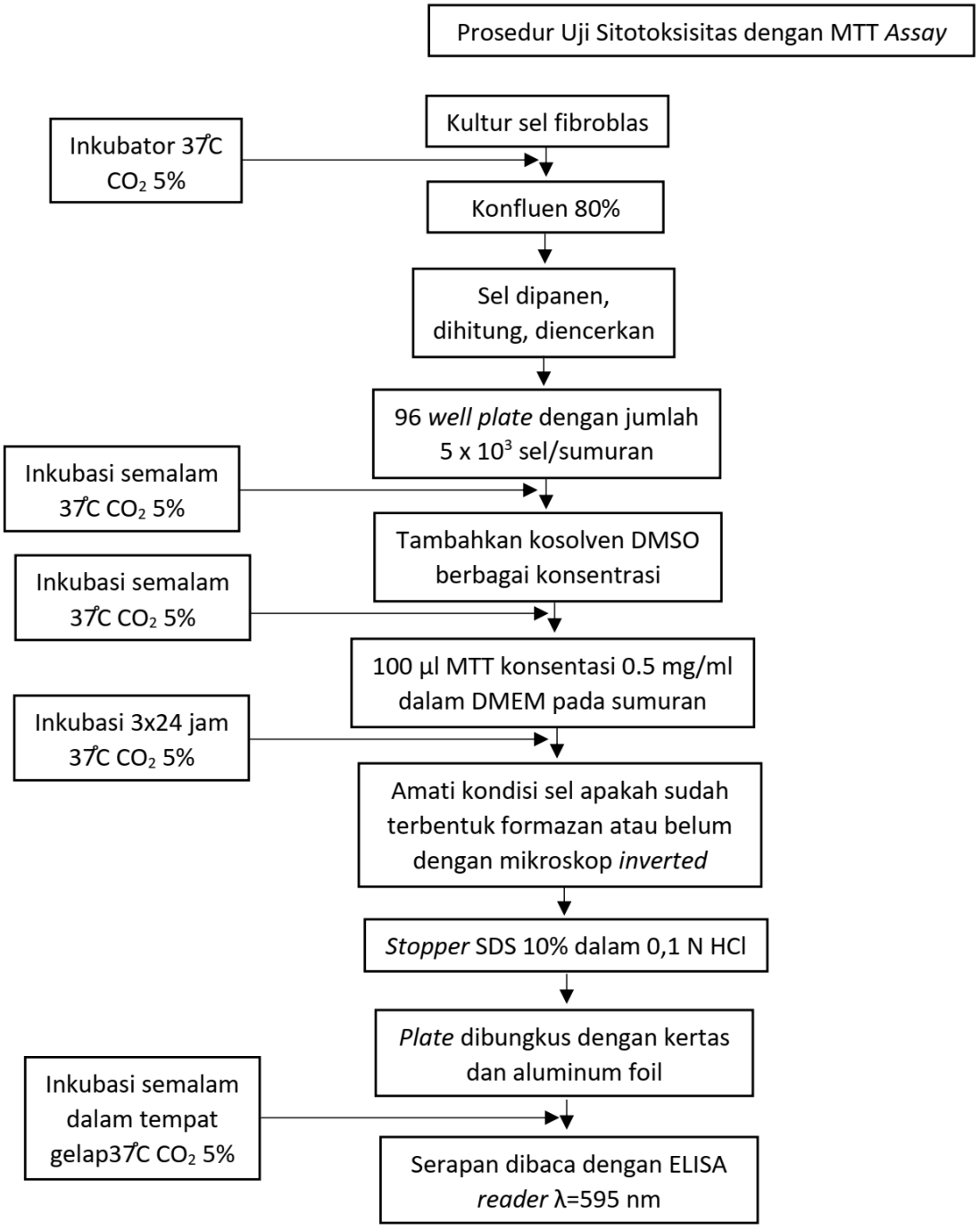


Gambar 3. Morfologi HA setelah dikalsinasi + GTA (sampel 4)

Pada tahun 2022 direncanakan akan dilakukan optimasi menggunakan DPSCs-CM, untuk itu perlu dilakukan terlebih dahulu uji toksisitas dan uji sterilisasi sinar Gamma.

Uji toksisitas menggunakan metoda MTT Assay dengan Elisa Reader yang dilakukan di *Dental Research Center* Fakultas Kedokteran Gigi Universitas Airlangga.

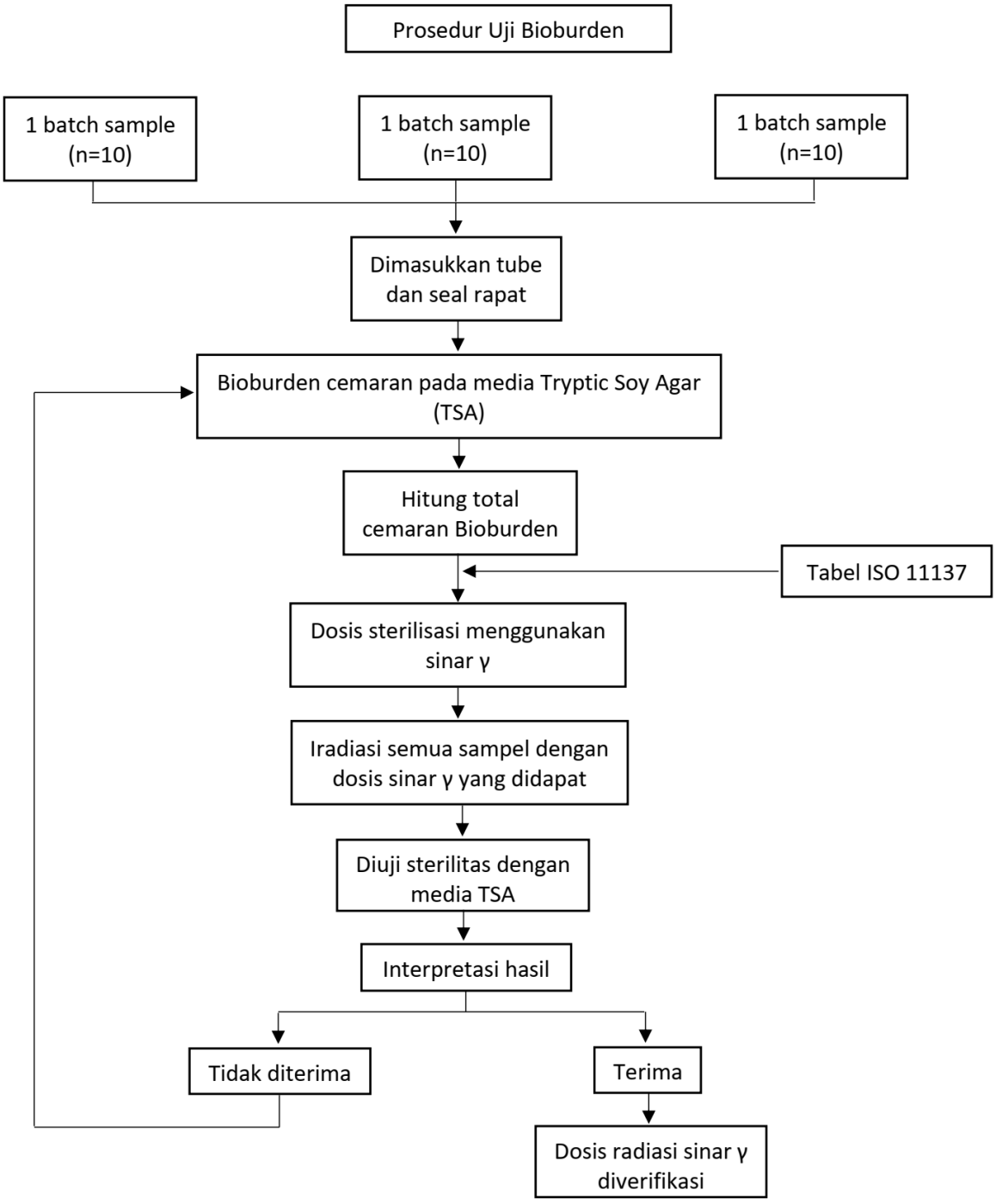
Alur penelitian:[28]



Uji Bioburden dilakukan untuk mendapatkan dosis efektif sinar Gamma. Dosis efektif sinar Gamma perlu diketahui terlebih dahulu agar dosis sinar Gamma yang digunakan untuk sterilisasi *bone graft* tidak terlalu besar karena akan merusak struktur *bone graft*; dan tidak terlalu kecil agar bone graft cukup steril untuk digunakan.

Uji bioburden dan sterilisasi sinar Gamma dilakukan di PT Energi Sterila Higiena yang memiliki fasilitas *Electron beam machine and X-Ray*. Kelebihan alat sterilisasi di PT Energi Sterila Higiena yaitu tidak menyisahkan residu sinar Gamma pada *bone graft* yag di sterilisasi. Hal ini penting karena residu sinar Gamma pada jumlah tertentu akan mempengaruhi *host*.

Alur penelitian.[29-31]



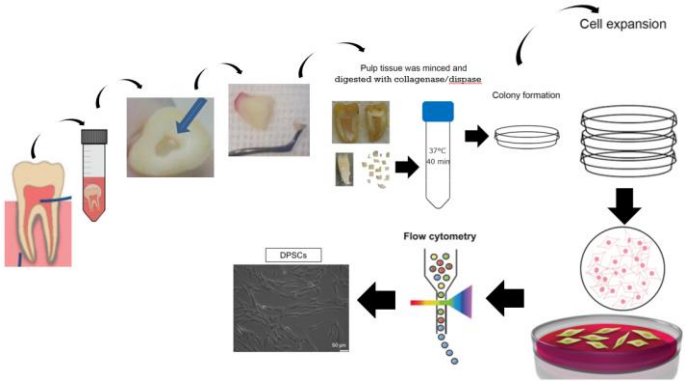
Optimasi BCP-Sr-Ag menggunakan DPSCs-CM.

1. Uji isolasi DPSCs
2. Karakterisasi DPSCs
3. Pembuatan DPSCs-CM
4. Perendaman BCP-Sr-Ag kedalam DPSCs-CM

1. Prosedur isolasi dan karakterisasi *dental pulp* dari gigi:

Prosedur isolasi gigi dengan menggunakan gigi M3 yang dicabut akibat proses impaksi total. Gigi yang telah diekstraksi diletakkan di dalam media transport pada suhu kamar atau pada suhu 4℃ dengan durasi paling lama 24 jam untuk dilakukan isolasi. Gigi dibelah dengan menggunakan bur untuk diambil jaringan pulpa. Jaringan pulpa yang telah diisolasi dilakukan pemotongan dan dicerna menggunakan enzim *collagenase/dispase* lalu diinkubasi dalam suhu 37℃ selama 40 menit sehingga terjadi pembentukan koloni. Koloni yang terbentuk kemudian dilakukan karakterisasi untuk memastikan koloni tersebut merupakan koloni *mesenchymal stem cells* (MSCs). CD73, CD90, CD105, CD34, dan CD45 digunakan untuk memastikan bahwa koloni yang diisolasi adalah DPSCs.

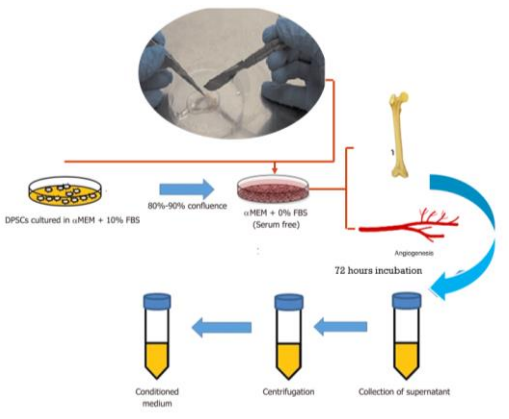
Alur prosedur isolasi dan karakterisasi *dental pulp stem cells* (DPSCs):



2. Prosedur pembuatan DPSCs-CM terdiri dari:

DPSCs yang telah diisolasi dan dikarakterisasi dikultur dalam media α-MEM yang disuplementasi dengan 10% *fetal bovine serum* (FBS) sampai tingkat konfluensi 80-90%. Setelah konfluensi 80-90% maka dilakukan proses re-kultur di dalam media α-MEM saja tanpa disuplementasi FBS namun disesuaikan dengan arah diferensiasi menuju ke DPSCs-CM osteogenik atau angiogenik. Dilakukan inkubasi selama 72 jam kemudian dilakukan sentrifugasi untuk mengumpulkan supernatant yang terbentuk dan didapatkan DPSCs-CM.

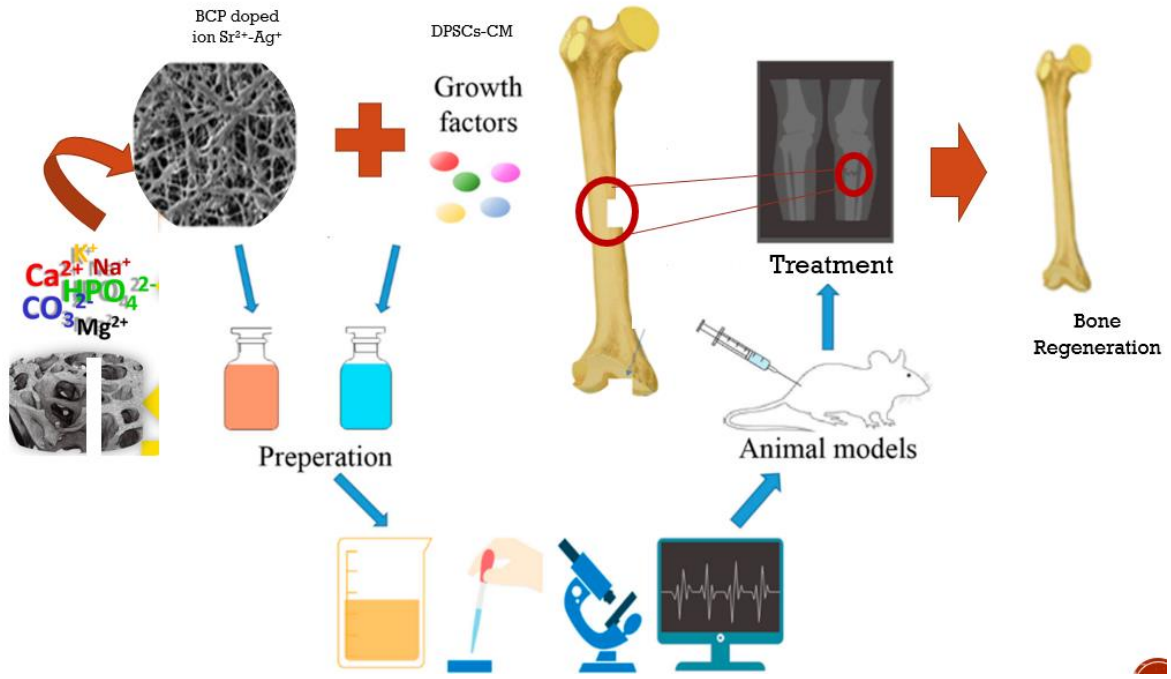
Alur prosedur pembuatan DPSCs-CM:



3. Prosedur Perendaman BCP-Sr-Ag kedalam DPSCs-CM:

BCP-Sr-Ag ke dalam DPSCs-CM dengan menggunakan metode perendaman *(immersion methode)*. Sampel BCP dikeringkan di dalam oven pada suhu 40℃ selama 24 jam lalu diambil sebanyak 10-gram dimasukkan ke dalam DPSCs-CM selama 24 jam sehingga BCP-Sr-Ag menyatu dengan DPSCs-CM.

Alur Perendaman BCP-Sr-Ag kedalam DPSCs-CM:



NO	AKTIFITAS	INDIKATOR	LUARAN	TAHUN PELAKSANAAN
1	Uji *mechanical properties* XRD, SEM dan *crystallinity*	Dokumentasi hasil SEM,XRD dan *crystalinity*	Journal (on going) Invited speaker	2021-2022
2	Uji Sitotoksisitas *bone graft* BCP-Sr-Ag menggunakan MTT *assay*	Sitotoksisitas *bone graft* BCP-Sr-Ag	Draft paten Paten registrasi Invited speaker Book manual	2022-2023
	Uji Bioburden *bone graft* BCP-Sr-Ag	Bioburden *bone graft* BCP-Sr-Ag		
	Studi in vitro *bone graft* BCP-Sr-Ag			
3	Uji in vivo optimasi *bone graft* BCP-Sr-Ag-DPSCs CM	Osteogenik dan angiogenik *induction* pada hewan coba	Paten Invited speaker Publikasi	2023-2024
4	Prototype optimasi *bone graft* BCP-Sr-Ag-DPSCs CM	Prototype	Prototype Book manual Jurnal Invited speaker	2024-2025

Keterangan:

Sudah dilakukan
Pengajuan proposal untuk 2022-2023
Pengajuan proposal untuk 2023-2024
Goal Ultimate

Susunan Organisasi Tim Peneliti dan Pembagian Tugas

No	NAMA/NIDN	INSTANSI ASAL	BIDANG ILMU	ALOKASI WAKTU (JAM/MINGGU)	URAIAN TUGAS
1	Dr. Dian Agustin W., drg.,Sp.KG(K) /00200871 02	Universitas Airlangga	Konservasi Gigi	15	Memimpin penelitian, draft paten,
2	Prof. Dr. Dwikora Novembri Utomo, dr., Sp.OT(K)/ 8897700016	Universitas Airlangga	Ortopedi dan Traumatology	10	Stem cell-CM
3	Dr. Hj. Khairul Anuar bin Shariff	Universiti Sains Malaysia	Bioceramic Engineering	10	Prototyping Uji material
4	Seling Nur Praduwana/non dosen	PT. ENERGI STERILA HIGIENA	Microbiology	10	Mitra Persiapan Komersialisasi Uji sterilisasi QC prototyping

Jadwal penelitian disusun dengan mengisi langsung tabel berikut dengan memperbolehkan penambahan baris sesuai banyaknya kegiatan.

JADWAL PENELITIAN

Tahun ke-1

No	Nama Kegiatan	Bulan											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Persiapan alat dan bahan												
2	Uji toksisitas												
3	Isolasi DPSCs												
4	Karakterisasi dan differensiasi DPSCs												
5	DPSCs-CM												
6	BCP-Sr-Ag-DPSCs CM												
7	Uji bioburden												
8	Analisis data												
9	Registrasi paten 1												
10	Buku Chapter dan luaran tambahan seminar												
11	Laporan kemajuan, deskripsi dan spesifikasi Paten												
12	Laporan akhir PTKN												

Tahun ke-2

No	Nama Kegiatan	Bulan											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Persiapan alat dan bahan												
2	Uji in vivo												

3	Pemeriksaan laboratoris												
4	Analisis data dan Dokumentasi pengujian paten												
5	Registrasi paten 2												
6	Publikasi/jurnal												
7	Buku manual dan luaran tambahan seminar												
8	Laporan kemajuan dan dokumentasi hasil uji coba Paten 1												
9	Laporan akhir PTKN												

Tahun ke-3

No	Nama Kegiatan	Bulan											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Persiapan alat dan bahan												
2	Prototype BCP-Sr-Ag-DPSCs CM												
3	Dokumen hasil uji coba paten												
4	Finalisasi paten/paten granted 1												
5	Buku Manual dan luaran tambahan seminar												
6	Laporan kemajuan												
7	Publikasi jurnal												
8	Laporan akhir PTKN												

Daftar pustaka disusun dan ditulis berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada usulan penelitian yang dicantumkan dalam Daftar Pustaka.

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Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. **HASIL PELAKSANAAN PENELITIAN:** Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian meliputi data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

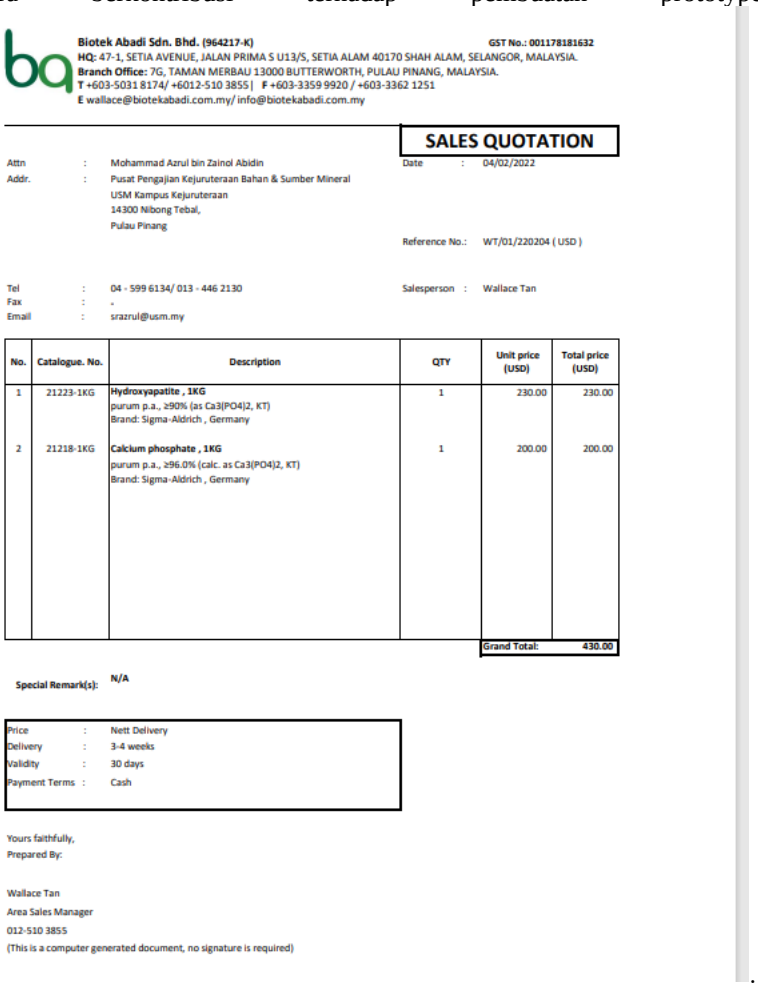
Pada tahun ini telah dihasilkan draft paten, dan telah dilakukan registrasi paten. ……

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui BIMA.

Paten registrasi dengan no S00202208736. Untuk manuscript sedang dilakukan pembuatan…

E**. PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (untuk Penelitian Terapan, Penelitian Pengembangan, PTUPT, PPUPT serta KRUPT). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui BIMA.

Mitra berkontribusi terhadap pembuatan prototype serta beberapa uji.



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Branch Office: 7G, TAMAN MERBAU 13000 BUTTERWORTH, PULAU PINANG, MALAYSIA.
T +603-5031 8174/ +6012-510 3855 | **F** +603-3359 9920 / +603-3362 1251
E wallace@biotekabadi.com.my/ info@biotekabadi.com.my

SALES QUOTATION

Attn : Mohammad Azrul bin Zainol Abidin
Addr. : Pusat Pengajian Kejuruteraan Bahan & Sumber Mineral
USM Kampus Kejuruteraan
14300 Nibong Tebal,
Pulau Pinang

Date : 04/02/2022
Reference No.: WT/01/220204 (USD)

Tel : 04 - 599 6134/ 013 - 446 2130
Fax :
Email : srazrul@usm.my

Salesperson : Wallace Tan

No.	Catalogue. No.	Description	QTY	Unit price (USD)	Total price (USD)
1	21223-1KG	**Hydroxyapatite , 1KG** purum p.a., ≥90% (as Ca3(PO4)2, KT) Brand: Sigma-Aldrich , Germany	1	230.00	230.00
2	21218-1KG	**Calcium phosphate , 1KG** purum p.a., ≥96.0% (calc. as Ca3(PO4)2, KT) Brand: Sigma-Aldrich , Germany	1	200.00	200.00
				Grand Total:	**430.00**

Special Remark(s): N/A

Price : Nett Delivery
Delivery : 3-4 weeks
Validity : 30 days
Payment Terms : Cash

Yours faithfully,
Prepared By:

Wallace Tan
Area Sales Manager
012-510 3855
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…

F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Manuscript terdapat kendala keterlambatan, karena ada uji yang diulang agar pembuatan draft paten bisa segera diselesaikan terlebih dahulu. Hal ini dilakukan karena paten merupakan luaran wajib …

G. **RENCANA TAHAPAN SELANJUTNYA:** Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

Meneruskan penelitian lebih lanjut untuk memperkuat prototype. Serta meneruskan publikasi yang terhambat…

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

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Osteoinduction Ability of Human Adipose-Derived Mesenchymal Stem Cell with Chitosan Scaffold Combination Towards Blood Serum Phosphorus Levels

Dian Agustin Wahjuningrum[1], Nindya R A Marhendra[2], M. Roelianto[1], Ari Subiyanto[1], Irmaleny[3], Fery Setiawan[4], Syania E Febriyanti[2], Setyabudi[1]*, Tamara Yuanita[1], Swadheena Patro[5], Anuj Bhardwaj[6,7]

1.Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
2.Resident of Department Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
3.Department of Conservative Dentistry, Faculty of Dentistry, Universitas Padjajaran, Bandung, Indonesia
4.Doctoral Student, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
5.Department of Conservative and Endodontics. Faculty of Dentistry. Kiit University. India
6.Adjunct Professor at Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
7.Department of Conservative Dentistry and Endodontics, College of Dental Science and Hospital, Rau, Indore.

Abstract

Reconstruction of extensive bone tissue damage is a treatment with complication. Moving the autologous tissue can cause problems in the repair of extensive tissue damage, so the principle of tissue engineering is used as an alternative to reconstruct damage to the tissue because it has many advantages. The combination of Human Adipose-Derived Mesenchymal Stem Cell (hADMSC) and chitosan scaffold is expected to trigger osteoinduction that can be expressed by osteogenic markers such as phosphorus levels in blood serum.

Objectives to prove osteoinduction in a combination of hADMSC and chitosan scaffold using blood serum phosphorus levels. This study used 12 groups with 5 sample each. Groups 1 to 4 were the negative control group at day 1,3,7, and 14. While groups 5 to 8 were the positive control group at day 1,3,7, and 14. Groups 9 to 12 were treatment groups at day 1,3,7, and 14. In the negative control group bone was only removed, in positive control group, bone was removed and chitosan scaffold was added, and in treatment group, bone was removed then, hADMSC and chitosan scaffold combination was added.

Blood collection will be carried out in each group for examination of phosphorus levels in the blood serum. There were differences in phosphorus levels in blood serum in each group even though statistically there were only significant differences on day 14. The combination of hADMSC and chitosan scaffold caused a significant change in blood serum phosphorus levels on day 14 which means it triggers osteoinduction.

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Introduction

Background

Tissue engineering is the application of the principles and methods of a homeostasis technique and science to a basic understanding of structural and functional relations in normal and pathological mammalian networks as well as the development of biological replacement to restore, maintain, or improve tissue function[1]. Tissue engineering is used in regenerative medicine which aims to repair and replace damaged or lost tissue by initiating natural regeneration processes such as osteoinduction which plays an important role in bone tissue regeneration[2,3].

Reconstruction of extensive bone tissue damage is one of the most difficult treatments for operators today[4]. The gold standard for improving bone repair is transplantation of fresh autologous bone[5]. However, when transferring autologous tissue such as bone graft it can cause complications because of the limited supply of

Corresponding author:
Setyabudi.
Faculty of Dental Medicine, Universitas Airlangga
St. Mayjen Prof. Dr. Moestopo No. 47, Surabaya, Indonesia
E-mail: setyabudi@fkg.unair.ac.id

tissue that causes problems with extensive tissue damage repair[4,5]. The principle of tissue engineering is used as an alternative to reconstruct damage to tissue because it has many advantages, namely, decreased donor morbidity, decreased procedural sensitivity in repair, and the ability to resemble in vivo tissue environments[4].

The principle of tissue engineering consists of three key elements namely stem cell, bioreactor/growth factor, and scaffold[1]. Stem cells are non-specialized cells that can regenerate themselves over long periods of time with the potential to be able to change into various types of tissues with certain functions[4]. Today it has been investigated that fat is not only an energy reserve but also a source of rich stem cells[6,7]. The benefits of hADMSC are readily available and easily accessed in the body with minimally invasive procedures which are also cheaper and adipose tissue is an operating waste[8,6]. In addition, hADMSC shows stable growth, extensive self-regenerative capacity and the ability to differentiate into various cell lineages such as osteogenic, adipogenic, chondrogenic, hepatogenic, and neurogenic cells, similar to human bone-marrow derived MSCs (BMSCs)[9,7]. In general, hADMSC appears to be a better choice for clinical applications than hBMSCs[10].

Furthermore, scaffold is used to become a template or framework of tissue formation and seeded with stem cells[1]. The selection of chitosan as a scaffold because chitosan has been widely used in bone tissue engineering, biocompatible, biomimetics and shows that chitosan increases cell growth and matrix deposition that rich in minerals by osteoblast cells[11]. In addition, the main source of chitosan is crustaceans or crab and shrimp shell waste that are widely found in Indonesia[12].

In this study, by combining hADMSC and chitosan scaffold, it can be used as an alternative to reconstruct damage to bone tissue by regenerating its original tissue. The presence of osteoinduction in combining the two ingredients, it is shown by several osteogenic markers such as, RUNX2 (run-related transcription factor 2) and phosphorus[13,14]. RUNX2 is osteoblast-related genes important in differentiating osteoblasts as well as chondrocyte maturation. RUNX2 induces chondrocyte maturation and increases chondrocyte proliferation through direct induction by expression of IHH (Indian Hedge Hog) which will induce PTHrP (Parathyroid-related Hormones Pathway)[13]. IHH and PTHrP will also express RUNX2 in the perichondrium and induce osteoblast differentiation[13]. Phosphorus or what can be called phosphate is one of the most abundant minerals in the human body and plays an important role in bone mineralization processes[14,15]. The serum phosphorus concentration is regulated by PTH (Parathyroid Hormones) which will increase phosphorus levels in blood serum during bone resorption[16]. Therefore, the osteoinduction of hADMSC with chitosan scaffold should be examined using RUNX2 markers as well as phosphorus levels in blood serum. This study was carried out in-vivo with a combination of adipose derived mesenchymal stem cells taken from human adipose tissue seeded in chitosan scaffold in the maxillary bone of wistar rats and examined using RUNX2 and phosphorus markers in blood serum as osteoinduction markers.

Materials and methods

Research Samples

The experimental animals used in this study were Wistar (Rattus norvegicus) rats, aged 3-month-old with an initial body weight of 300-330 gr, obtained from Gajah Mada University, Yogyakarta, Indonesia. The Wistar rats (Rattus norvegicus) were housed in a cage made of plastic tub and given a cage cover made of fence wire. Rat cages are conditioned in clusters that are placed in a place with equal temperature and humidity between groups. Rats are kept and adapted for one week in a cage with free access to food and water.

Research Methods

1. Sample preparation stage

Experimental animals are randomly selected and divided into 12 groups: The K (-) 1 group is the negative control group as normal bone which is drilled. Phosphorus levels in blood serum were checked on day one, The K (-) 3 group is the negative control group as normal bone which is drilled. Phosphorus levels in blood serum were checked on the day three, The K (-) 7 group was the negative control group as a normal bone which is drilled. Phosphorus levels in the blood serum were checked on the day seven, The K (-) 14 group was the negative

control group as a normal bone which is drilled. Phosphorus levels in blood serum were checked on day fourteen. The K (+) 1 group is the positive control group with the administration of chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on day 1. The K (+) 3 group was the positive control group with the administration of chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on the day three. The K (+) 7 group was the positive control group with the administration of chitosan scaffold after bone drilling. Phosphorus levels in the blood serum were checked on the day seven. The K (+) 14 group was the positive control group with the administration of chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on day fourteen. The treatment group P1 is the group with the administration of hADMSC on chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on day one. The treatment group P3 is the group with the administration of hADMSC on chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on the day three. The treatment group P7 is the group with the administration of hADMSC on chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on day seven. The treatment group P14 is the group with the administration of hADMSC on chitosan scaffold after bone drilling. Phosphorus levels in blood serum were checked on day fourteen.

2. Human Adipose-derived Mesenchymal Stem Cell isolation and culture

Freshly taken lipoaspirate and washed with PBS 1% sterile until golden in color. The adipose tissue was then dissolved with a solution of 0.01% collagenase or made in PBS solution with a ratio of 1 ml of enzyme solution and 1 cm^3 of adipose tissue. This mixture then incubated at 37° C with intermittent agitation until it is completely mixed (usually 30 minutes). Infranatant were then carefully suctioned, transferred in 50 ml conical tubes and centrifuged at 706 x g for 8 minutes with PH 7.3. The supernatant was removed and produced a pellet SVF, resuspended to the media control of Dulbecco's Modified Eagle's Medium Low Glucose (DMEM-LG) supplemented with 10% serum bovine fetal (FBS), penicillin 500 IU and streptomycin 500lg (Mediatech, Manassas, VA, USA). Cells were then counted on T75 flasks that were not coated with a concentration of 1 x 10^6 cells. Then 20 mg lipoaspirate is sufficient tissue to produce adequate SVF (> 1 x 10^7 cells)[22]. Mononuclear cells were coated in expansion medium (MI) at a density of 10^5 cells / cm2 in tissue culture coated with 10 ng/ml fibronectin (Sigma, Deisenhofen, Germany). Media expansion consists of 58% Dulbecco's Modified Eagle's Low Glucose Medium (DMEM-LG, Cambrex, Apen, Germany) and 40% MCDB201 (Sigma), 2% fetal calf serum (FCS; StemCell Technologies, Vancouver, BC, Canada), equipped with 2mM L-glutamine, 100 U / ml Pena / Strep (Gibco, Enggestein, Germany), 1% insulin transferrin selenium, 1% linoleic acid bovine serum albumin, 10 nM dexamethasone, and 0.1 mM L-ascorbic acid-2 -phosphate (all from Sigma), platelet-derived growth factors, and epidermal growth factors (10 ng / mL each). When it reaches 80% confluence, cells are tested with 0.25% trypsin / 1 mM EDTA (Invitrogen, Karlsrube, Germany) and replaced at around 9,000 cells / cm^2, cells are expanded for 2 to 6 passages[23].

Then, we identify hADMSC with the phenotypic mesenchymal stem cell (MSC) kit after the second passage. hADMSC on the expressions of CD73, CD90, and CD 105 has no expression also in expression of CD45, CD34, CD14, or CD11b, CD 79a, and HLA-DR. Cells were analyzed using Accurl C6 Flow cytometer (BD Biosciences, San Jose, CA, USA) which showed positive staining for CD90 (81.3%), CD105 (86.6%), and CD73 (99.9%) and negative coloring for CD14, CD20, CD34, and CD45. After that, these cells can be identified in the adipogenic, osteogenic or chondrogenic conditions available in the kit (Cyagen Biosciences Inc., Sunnyvale, CA, USA).

3. Chitosan scaffold toxicity test for hADMSC

Toxicity test of chitosan scaffold on hADMSC cell culture was carried out with trypsination from one petri plate which covered 2.5 x 10^6 cells. Then resuspension into DMEM / F12 medium and centrifugation. Pellets were grown into culture with 96 wells (M96) each of 5 x 10^4 cells/wells then incubated for 24 hours at 37°C and 5% CO_2. When 80% of the growth is obtained, chitosan scaffold inserted into ½ parts of the well that have contained cells. DMEM / F12 medium was added as much as 100 microliters, then incubated again for 20 hours at 37°C and 5% CO2. After that, MTT was added 5

mg/mL (25 microliters / well), then incubated for 4 hours and watch under the inverted microscope. Scaffold and medium removed then added DMSO 200 microliter/well. Each the well read by Elisa Reader at a wavelength of 595 nm.

4. hADMSC seeding in chitosan scaffold

Cell suspension containing $5x10^5$ cells in 100 µl medium was poured on the surface of each scaffold placed on unprocessed six-well non-tissue culture plates. Each construct was incubated at 37° C for 1 hour. After 1 hour, 4.5 ml of chondrogenic media are added to each well. Plates are placed on an oscillating shaker platform 60 rpm. Construction was maintained at 37° C with 5% CO2 in chondrogenic media for 72 hours, 10 days or 21 days. The medium is changed three times a week.

5. Transplantation of hADMSC and chitosan scaffold into bone defect

Anesthetic injection is performed using ketamine HCl 50 mg/Kg body weight and xylazine HCl 10 mg/kg intramuscularly in the femoral caudal extremity. Anesthetized rats are shaved in the ventral hair with a dorsal recumbency position. Antiseptic application is applied using cotton containing kalium iodide and applied to shaved rats' skin. The incision is carried out using a blade on the superficial skin of the maxillary bone, approximately 1 cm under the eye. After the incision is successful and the maxillary bone is visible, the maxillary bone is drilled using a slow-speed hand piece with 1500 rpm speed to 3mm lateral and 2mm profundus. During drilling, sterile saline solution is given. Transplantation of chitosan scaffold containing hADMSC in rats were carried out after drilling completed. Positive control groups were drilled and planted with scaffold whereas, the negative control group were only drilled and administrated of sterile saline solution. Bone closure is done by suturing on 2 layers of tissue. After suturing in the first layer, intraperitoneal enrofloxacin was injected. The sutured skin is given an antiseptic on the surface of the suture and is given a sterile gauze and covered with surgical tape plaster and bandaging with wound tape.

6. Phosphorus serum study

All groups of experimental animals were given uniform standard feed during adaptation until the end of the treatment. On day one, three, seven and fourteen, experimental animals were sacrificed to take the maxilla. Control group and treated group rats were anesthetized using ketamine HCl at a dose of 50 mg/kg. After the rats did not show a painful response, the rat was laid down on top of the dissecting tray. Each leg is fixed with a needle. All skin and hair are moistened. The opening of the abdominal space is done by means of the abdominal muscles (abdominal wall) being cut in linea alba starting from the tip of the breastbone (process xiphoideus) to the pubic pectin ossis. After opening the abdominal wall, the chest cavity is opened, with the last rib cut to the front toward the sternal bone arch. Cutting is done on the right and left side until the heart is visible so that blood collection using a syringe through the right atrium of the heart can be done. Then, we centrifuge the blood to get blood serum. Blood phosphorus level were examined using UV-VIS method.

Statistical methods

Data of all phosphorus levels were firstly analyzed using the One-Sample Kolmogorov-Smirnov test to find out the normal distribution using p value = 0.05. After checking the normality test all the groups had $P<0.05$ so that oneway Anova statistical test can be done to find out the significant difference using p value = 0.05. The phosphate on the day one, three, and seven were insignificant with $P>0.05$, but the phosphate on the day fourteen was significant with $P<0.05$. Thus, it can be stated that there was a significant difference data in the phosphate level on the day fourteen among the day one, three, and seven.

Results

This study was conducted *in-vivo* with the combination of human adipose derived mesenchymal stem cells taken from human adipose tissue seeded in chitosan scaffold in the maxillary bone of wistar rats that have been drilled. The chitosan scaffold toxicity test for human adipose derived mesenchymal stem cells using the MTT assay method was carried out at the Airlangga University Stem Cell Research Center. Observation of the ability of combined osteoinduction of human adipose derived mesenchymal stem cell and chitosan scaffold was carried out in 12 groups by measuring the phosphorus levels in rat blood serum from each sample and expressed in units (mg/dl) in tables 1, 2, 3, and 4.

Sample	Day One		
	Negative Control (K (-) 1) (mg/dl)	Positive Control (K (+) 1) (mg/dl)	Treatment (P1) (mg/dl)
1	7	9	9.1
2	7.5	9.1	10.9
3	7.9	7.2	6.7
4	8	5.3	8.7
Mean	7.6	7.65	8.85

Table 1. Phosphorus levels in blood serum in day one.

On the first day (table 1), the average number of phosphorus levels with the difference in each group was 7.6 mg/dl in the negative control, 7.65 mg/dl in the positive control and 8.85 mg/dl in the treatment. On the third day (table 2), the number of phosphorus levels are higher in the positive control group was 8.6 mg / dl, whereas in the negative control the phosphorus level was 6.725 mg/dl and the treatment showed phosphorus levels of 6.6 mg/dl.

Sample	Day Three		
	Negative Control (K (-) 3) (mg/dl)	Positive Control (K (+) 3) (mg/dl)	Treatment (P3) (mg/dl)
1	6	8.3	4.8
2	6.8	8.6	7
3	6.2	6.2	6.7
4	7.9	9.2	7.9
Mean	6.725	8.6	6.6

Table 2. Phosphorus levels in blood serum in day three.

Sample	Day Seven		
	Negative Control (K (-) 7) (mg/dl)	Positive Control (K (+) 7) (mg/dl)	Treatment (P7) (mg/dl)
1	9.7	9	8.2
2	8.8	8.8	8.8
3	9.7	6.6	8.4
4	9.4	7	6.6
Mean	9.4	7.85	8

Table 3. Phosphorus levels in blood serum in day seven.

Sample	Day Fourteen		
	Negative Control (K (-) 14) (mg/dl)	Positive Control (K (+) 14) (mg/dl)	Treatment (P14) (mg/dl)
1	8	8.4	6.4
2	8.6	6.8	9
3	9.8	5.6	7
4	9.6	6.4	6
Mean	9	6.8	7.1

Table 4. Phosphorus levels in blood serum in day fourteen.

On the day seven (table 3) there was a higher number of phosphorus levels in the negative control ie 9.4 mg/dl. In the positive control, phosphorus levels were 7.85 mg/dl and phosphorus levels were 8 mg/dl. Then, on the day fourteen (table 4) found that the phosphorus level in the negative control was 9 mg/dl, in the positive control there was a 6.8 mg/dl phosphorus level, and the treatment was 7.1 mg/dl phosphorus.

	Group	Mean ± SD	P value
Phosphate day one	Negative Control (K (-) 1)	7.6 ± 0.45461	0.426
	Positive Control (K (+) 1)	7.65 ± 1.79351	
	Treatment (P1)	8.85 ± 1.72337	
Phosphate day three	Negative Control (K (-) 3)	6.725 ± 0.85391	0.199
	Positive Control (K (+) 3)	8.0750 ± 1.30480	
	Treatment (P3)	6.6 ± 1.30384	
Phosphate day seven	Negative Control (K (-) 7)	9.4 ± 0.42426	0.082
	Positive Control (K (+) 7)	7.85 ± 1.22610	
	Treatment (P7)	8 ± 0.96609	
Phosphate day fourteen	Negative Control (K (-) 14)	9 ± 0.84853[a]	0.046
	Positive Control (K (+) 14)	6.8 ± 1.17757[b]	
	Treatment (P14)	7.1 ± 1.33167[c]	

Table 5. Mean and standard deviation of each group based on phosphate day one, three, seven, and fourteen. The different superscript letters are statistically significant (Tukey HSD, $P < 0.05$).

Table 3 shows that the negative control group on the day seven showed the highest serum phosphorus level compared to the other groups, while the lowest serum phosphorus level was the treatment group the day three in table 2.

Data from this study in the form of phosphorus levels need to be analyzed first using the One-Sample Kolmogorov-Smirnov test to find out the normal distribution of the results of the study and the test of homogenity of variances to determine the homogenity of the results of the study. After doing the two statistical tests, oneway Anova statistical test can be done to find out the differences between sample groups. Based on the results of the normality of serum phosphate levels using the one sample Kolmogorov-Smirnov Test, it is known that the data is normally distributed because it has significance value > 0.05. Based on the test results of homogenity of serum phosphate levels,

it is known that the overall data is homogeneous because it has significance value > 0.05.

Oneway Anova test was used to determine the significant differences in all groups. From this test, the value of P or significance will be obtained between groups and all groups in table 5. On the day one, day three, day seven showed a p value greater than the significance value (p> 0.05) so that the phosphate level can be concluded on the day one, three and seven were not significant. However, on day fourteen, the test results showed a p value smaller than (p <0.05) which could be concluded that the phosphate level on day fourteen had a significant value between groups.

Discussion

This study was conducted *in vivo* to prove the presence of changes in phosphorus levels in blood serum as an indication of osteoinduction after transplantation of Human Adiposed Derived Mesenchymal Stem Cell (hADMSC) with chitosan scaffold on wistar rat maxillary bone defects on day 1,3,7, and 14. Use of Human Adiposed Derived Mesenchymal Stem Cell in accordance with Aksu (2008) which states that Human Adiposed Derived Mesenchymal Stem Cell can differentiate into osteoblast like cells[17]. The use of Chitosan as scaffold in this study is based on Dash (2011) research which states that chitosan increases cell growth and rich minerals matrix deposition by osteoblast cells[11]. So that the combination of the two ingredients will induce an osteoinduction pathway, specifically PTHrP and Indian Hedgehog that will increase RUNX2 to convert hADMSC into osteo-chondroblast progenitors which will later become pre-Osteoblasts. To begin bone remodeling, pre-osteoblast can go through two paths, namely osteoclast formation influenced by PTHrp or the formation of mature osteoblasts which will increase OPG so that it will affect phosphorus levels in blood serum.

From the results of the study conducted an oneway anova test to see statistically whether there were significant differences in results, from statistical analysis showed that the ratio of phosphorus levels in the blood to negative controls, positive control and treatment group on the first, third and seventh days there were no significant differences, because the p value (Sig) shows a number above 0.05. Significant differences between treatment groups were only seen on day fourteen, because the value of p (Sig) showed a number below 0.05.

On the first day, there was no significant difference in the level of phosphorus in the blood because the bone healing process that occur on the first day was an inflammatory process where there was no significant process of resorption and bone mineralization which could affect blood phosphorus levels according to Mountziaris (2008)[18]. But in the results of the study, there were still differences in phosphorus levels from each group, with an mean level of phosphorus in treatment group higher than positive controls and negative controls. The high level of phosphorus in the treatment group can occur because of several possibilities including, dietary phosphate intake is higher than other groups and there is more increase in PTH compared to other groups because the number of Mesenchymal Stem Cells in the treatment group is higher than other groups so that it will increase absorption of phosphate in the intestine[14]. Increased PTH originates from an increase in Ihh-PTHrp axis activity that induces RUNX2 to include hADMSC into the osteoblastic line and osteogenic differentiation of hADMSC which also occurs on the first day of bone healing[19].

Then, on the third day there was also no significant difference in blood phosphorus levels because the bone healing process that occurred was hADMSC proliferation and differentiation of preosteoblasts into osteoblasts which did not significantly affect serum phosphate in the blood according to Mountziaris (2008) and Dimitriou (2005) [18,20]. However, in the results of the study, the lowest levels of phosphorus were found in the treatment group compared to negative controls which might be due to lower dietary phosphate intake compared to other groups and osteogenic differentiation of hADMSC which requires PTH so that PTH decreases which will reduce absorption calcium and phosphate in the intestine[16]. The highest phosphorus levels were found in positive controls which might also caused by changes in the key phosphate regulation in accordance with Penido and Alon (2012) , which are higher dietary phosphate intake than other groups and changes in hADMSC into osteoblastic lines and possible osteogenic differentiation still occurs on day 3 according to Li's study (2015) so that PTH production is still relatively more than normal and can increase phosphate regulation in serum [16, 21].

On the seventh day, there was no significant difference in the level of phosphorus in the blood serum because the normal bone healing process, which occurred on the seventh day was the continuation of the same process from the third day namely the peak of MSCs cell proliferation in intramembranous ossification. So that at this stage there is little effect on phosphate levels in blood serum. From the results of the seventh day study, phosphorus levels in the positive control and treatment were lower than the phosphorus levels in the negative control, which can occur due to lower phosphate dietary intake and the process of changing osteogenic differentiation of hADMSC that requires PTH to be completed.

On the fourteenth day, there is a significant or significant difference in the level of phosphorus in the blood serum. This is because the fourteenth day is the most active phase of osteogenesis until the 21st day is characterized by the cessation of cell proliferation in intramembranous ossification, mineralization of soft callus, cartilage resorption, formation of woven bone, and the beginning of the bone remodeling phase that most affects phosphorus levels in the blood because of the mineralization and resorption processes according to Mountziaris (2008) and Dimitriou (2005)[18,20]. Based on the results of the study, the level of phosphorus in the blood serum in the positive control and treatment was significantly lower than the negative control due to the higher mineralization ratio in the positive control and treatment according to Penido and Alon (2012)[16]. which stated that the increased mineralization would decreases serum phosphorus and calcium concentration, and increased bone resorption will increase serum phosphorus and calcium concentrations. In addition, the low level of phosphorus in blood serum is also due to the increasing number of mature osteoblasts which will allow OPG to inhibit osteoclast function for bone resorption according to the statement of Deschaseaux (2009)[19] so that bone mineralization processes can increase. FGF23 or Phosphatonin which is widely expressed by osteoblasts and osteocytes will also reduce PTH so that it will improve bone mineralization processes in accordance with the statements of Penido and Alon (2012) [16,19].

Conclusions

In conclusion, based on the results of the analysis, the combination of hADMSC and chitosan scaffold caused a significant change in blood serum phosphorus levels on day 14 which means it triggers osteoinduction.

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Declaration of Interest

The authors declare no conflicts of interest.

Ethical policy and institutional review board statement

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya ((Permit Number: 217/HRECC.FODM/VIII/2018) in 2018.

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Levels of Wistar Calcium Serum (*Rattus norvegicus*) in Human Adipose-derived Mesenchymal Stem Cells (hADMSCs) and Chitosan Scaffold by Osteoinduction Examination

Dian Agustin Wahjuningrum[1]*, Setyabudi[1], Destri Imania[2], Ria Chusnita[2], Andi Syahrimayani[2], Latief Mooduto[1], Anuj Bhardwaj[3,4]

1. Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
3. Department of Conservative Dentistry and Endodontics, College of Dental Science and hospital, Indore, India.

Abstract

Bone tissue reconstruction with extensive damage is one of the challenges for dentists because its healing process of bone tissue. Bone graft is the gold standard for bone repair. However, the use of bone graft has a limited amount of tissue produced. Tissue engineering is the latest method in terms of bone regeneration. Tissue engineering has three main components, stem cells that have self-renewal ability and multilineage differentiation, bioreactor / growth factor, and scaffold. The combination of hADMSCs and chitosan scaffold, is expected to trigger osteoinduction shown by osteogenic markers such as calcium levels.

Objectives to prove osteoinduction activity in a combination of Human Adipose-derived Mesenchymal Stem Cell (hADMSCs) and chitosan scaffold using serum calcium levels.

There were 12 treatment groups with each group having 4 samples. Groups 1 to 4 were the negative control group at day one, three, seven, and fourteen which maxillary bone drilling only. While groups 5 to 8 were the positive control group at day one, three, seven, and fourteen which were given chitosan scaffold. Groups 9 to 12 were treatment group at day one, three, seven, and fourteen which were given hADMSCs and chitosan scaffold. Blood is collected in each group to check serum calcium levels. There were differences in calcium levels in blood serum in each group.

The application of hADMSCs and chitosan scaffold caused a significant change in serum calcium levels on the day one, three, seven, and fourteen which meant that the combination of hADMSC and chitosan scaffold could trigger osteoinduction.

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Introduction

Extensive reconstruction of bone tissue requires a bone graft. Autografts are "Gold standards" that are used for bone tissue reconstruction to date. However, autografts have only produce limited number of cell.[1] So, new method is needed to augment extensive bone defects.

Tissue engineering is the latest method of regenerating tissue without leaving scar.[2] Tissue Engineering has three main components, cells, scaffold, growth factor or bioreactor.[3,4,5] Stem cells have the ability to self renewal and multilineage differentiation.[6] Bone marrow mesenchymal stem cells (BMSCs) is a source of stem cells commonly used in tissue engineering.[7] BMSCs are able to differentiate into chondrocytes, osteoblasts, adipocytes, fibroblasts and other mesenchymal tissues.[8] However, BMSCs produce a low number of stem cells, require more in vitro time, painful aspiration procedures, poor proliferation and cell osteogenic abilities are influenced by the age of the patient. The source of new stem cells is found from fat tissue, so-called adipose-derived stem cells (ASCs) and when obtained from humans it is called human adipose-derived mesenchymal stem cells (hADMSCs).[9] hADMSCs are considered more suitable in clinical applications because the number of stem cells produced through lipo-aspiration is numerous, cell

Corresponding author:
Dian Agustin Wahjuningrum,
Faculty of Dental Medicine Universitas Airlangga
St. Mayjend. Prof. Dr. Moestopo No. 47, Surabaya, Indonesia.
E-mail: dian-agustin-w@fkg.unair.ac.id

proliferation is fast, aspiration procedures are more comfortable and death during lipoaspiration is lower.[10,11] However, the osteogenic capacity of hADMSCs is not greater than that of BMSCs.[12] Therefore, a material is needed to increase the osteogenic capacity of hADMSCs.

Bone regeneration requires osteoconductive, osteoinductive scaffold while providing a suitable microenvironment for proliferation, differentiation and tissue formation.[13] Some of the scaffolds used today are collagen, chitosan, gelatin, alginate, fibrinogen, and hyaluronic acid.[14] Chitosan is chosen as scaffold because chitosan has biocompatible properties, can mimic the original network microenvironment and chitosan can increase cell growth and mineral deposition of minerals by osteoblast cells.[15] In addition, chitosan scaffold also has a structure similar to glycosaminoglycan. Glycosaminoglycan is one of the main components connected with collagen fibers in the extracellular matrix (ECM).[16]

Objectives to explain bone osteoinduction activity that can be seen by osterix (OSX) expression and serum calcium level as evidenced by the active mechanism of sending induction stem cell signals to differentiate into pre-osteoblasts.[17]

Materials and methods

Research Samples

This study used a sample of chitosan scaffold in the form of membrane with porosity 25 taken from stock at BATAN and hADMSCs taken from human adipose tissue at the Airlangga University Stem Cell Research and Development Center. Male wistar (Rattus Norvegicus) rats aged 3 months with a body weight of 300-330 grams as many as 48 birds with healthy conditions were obtained from UD. Tiput Abadi Jaya. The sample size in each group was calculated using the Federer formula and obtained at least 3 rat samples each group.

Research Methods

Anesthetic injection using ketamine HCl 50 mg / Kg BB and xylazine HCl 10 mg / Kg BB intramuscularly in the femoral caudal extremity. Then maxillary bone drilling was done, 1 cm below the eye using a low-speed hand piece operated 1500 rpm lateral to 3 mm long and profundus as long as 2 mm. During drilling sterile saline solution was given.

In the first group, the negative control group only performed maxillary bone drilling and were not given hADMSCs or chitosan scaffold but saline was added during drilling. The second group was the positive control group performed maxillary bone drilling and was given chitosan scaffold after bone drilling and addition of saline during drilling. The third group, the treatment group, carried out maxillary bone drilling by adding hADMSCs on chitosan scaffold after bone blast and adding saline during drilling.

On the day one, three, seven, and fourteen 2 cc of blood was collected in the aorta. Next, blood was put into a centrifugation tube and centrifuged 1500 rpm for 20 minutes to obtain serum. The serum is labeled according to group and stored in a deep freezer at -70^{o}C until all serum samples are collected. Measurement of calcium levels is carried out by the principle of calorimetry method O-cresolphthalein complexone. A serum sample of 1 ml piped into the test tube was then added with 4 ml of 5% TCA. The solution is cortexed (homogenized), then centrifuged at 3000 rpm for 30 minutes. The resulting supernatant was pipetted 1 ml each into the test tube, then added a solution of strontium (Sr) 5% as much as 1 ml and added as much as 8 ml of distilled water. After that, it was analyzed by a spectrophotometer at a wave of 422.4 nm. The standard calcium solution used is calcium carbonate ($CaCO_3$). The reading results are then compared to the standard curve, so that calcium levels are obtained in units of mg / dl or ppm 28.

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya (No. 255 / HRECC.FODM / IX / 2018) on 18 September, 2018.

Statistical methods

The results of the next study were analyzed statistically by one way ANOVA and continued with the Tukey HSD test with a significance value of $p < 0.05$.

Results

The results showed that calcium levels changed in the treatment group when compared to the negative control group and the positive control group both on day one, three, seven, and fourteen.

On the day one serum calcium levels in the treatment group had the lowest value compared

to the negative control group and the positive control group (Table 1).

	Group	Mean ± SD	F	P value
Day one	Negative Group[a]	10.2075 ± 0.11587	59.548	0.000
	Positive Group[b]	12.05 ± 0.58023		
	Treatment Group[c]	9.4775 ± 0.963		
Day three	Negative Group[a]	9.99 ± 0.207	45.438	0.000
	Positive Group[b]	11.85 ± 0.591		
	Treatment Group[c]	9.4375 ± 0.170		
Day seven	Negative Group[a]	9.59 ± 0.146	18.868	0.001
	Positive Group[b]	12.05 ± 0.619		
	Treatment Group[c]	11.65 ± 0.838		
Day fourteen	Negative Group[a]	9.92 ± 0.294	113.519	0.000
	Positive Group[b]	12.75 ± 0.12910		
	Treatment Group[c]	12.575 ± 0.40311		

Table 1. Mean, standard deviation (SD), and p value of serum calcium of the negative, positive, and treatment group. The different superscript letters are statistically different (ANOVA, $P < 0.05$).

Whereas on the day three, the serum calcium level in the negative group and the treatment group had an average value that was not far adrift. The mean serum calcium levels in both the negative, positive and treatment groups on the third day decreased slightly compared to the day one (Table 1).

On the day seven the mean of negative, positive and treatment groups are increased compared to the day three. The serum calcium level of the treatment group has the highest increase compared to the negative control group and the positive control group (Table 1).

On the day fourteen, the mean serum calcium levels in the negative, positive, and treatment groups increased compared to the day seven (Table 1).

The results of one way ANOVA test in this study found a significance value of $p = 0.000$ ($p < 0.05$) which means the comparison of serum calcium levels in each group in the negative control group, the positive control group and the treatment groups day one, three, seven, and fourteen were the difference with the value of $p = 0.000$ ($p < 0.05$). Furthermore, to find out further differences between groups, the Tukey HSD test was conducted. Tukey HSD results showed that there were significant differences between groups in both the negative control group, the positive control group and the treatment group (Table 1).

Discussion

Tissue engineering is a cutting-edge strategy that aims to restore the function of the tissue that has a defect. Tissue engineering has three components, namely growth factor or bioreactor, biomaterial scaffold, and cell. Absolute bone regeneration process requires osteoconductive, osteoinductive scaffold while providing a suitable microenvironment for proliferation, differentiation and tissue formation.[18]

On the day one of negative control, serum calcium levels increased due to the inflammatory process. Bone fractures are known by the body as stressors. In the brain, stressor is translated as a host defense response which then stimulates the hypothalamus and activates the hormonal system of the HPA (Hypothalamic-pituitary-adrenal) axis. Activation of the HPA axis pathway will stimulate the hypothalamus to secrete corticotropic releasing hormone (CRH). CRH stimulates the anterior pituitary to secrete ACTH (adrenocorticotropic hormone) then ACTH will trigger the adrenal cortex to secrete glucocorticoid hormones. One of the glucocorticoid hormones secreted when stress occurs is corticosterone. Increased corticosterone levels will increase the activity of inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)[19,20] which activate osteoclasts. Then osteoclasts will resorb bone resulting in extracellular calcium release. The release of calcium to extracellular minerals will have an impact on increasing serum calcium levels1.[21]

Immediately after a bone fracture, hematoma formation results from rupture of a blood vessel. Tissue in the fracture area will experience hypoxia due to reduced blood supply due to damage to blood vessels[22] which then encourages bone resorption due to a decrease in PTH secretion (Parathyroid hormone). PTH is an important hormone secreted by the parathyroid gland which functions to regulate blood calcium levels.[23]

The decrease in PTH on the day one is likely due to bone immobilization or hemiplegic

stroke[24] which can cause increased bone resorption, decreased bone formation and increased serum calcium levels.[23,25]

The mean serum control serum calcium level had the lowest value compared to the negative control group and the positive control group because mesenchymal stem cells could be induced to become osteoblasts if there were inflammatory cytokines such as IL-1, IL-6 and TNF-α.[26,27] Thus, on the first day of acute inflammation where IL-1 IL-6 and TNF-α activity was high, the treatment group given hADMSCs and chitosan scaffold could more quickly induce mesenchymal stem cells to become osteoblasts. Besides mesenchymal stem cells can produce osteoprotegerin which serves to suppress RANKL / RANK pathways so that the osteoclastogenesis process is inhibited[28] and extracellular Ca^{2+} decreases. The decrease in extracellular Ca^{2+} results in a decrease in serum calcium levels.

Then on the day three proliferation and differentiation of pre-osteoblasts becomes osteoblasts. The activity of IL-1, IL-6 and TNF-α was also suppressed so that the production of osteoclasts decreased and bone resorption also decreased but the inflammatory process still occurred.[6] This is what causes the serum calcium level on the day three to decrease compared to the day one. In the treatment control, serum calcium levels decreased slightly compared to the day one because of the inflammatory process that still occurs so that the presence of IL-1, IL-6 and TNF-α in bone healing process causes mesenchymal stem cells hADMSCs to bind IL-1, IL-6 and TNF -α through the WnT pathway signaling and osteoclast production decreases.[29,30]

On the day seven the mean serum calcium level increases compared to the third day which means extracellular Ca^{2+} rises. This is because on the seventh day is the peak of TGF-β 6 which functions to increase proliferation, angiogenesis and formation of connective tissue and soft callus via intramembran and endochondral ossification by inhibiting the activity of osteoclasts.[15,31,32] Meller et al., Also explained that increased PTH levels were seen during the formation of soft callus as compensation for the body to mobilize calcium from bone as a callus precursor mineralization.[33,34] So that when PTH levels increase, calcium in plasma levels also increase because PTH decreases osteoclast activity, increases osteoblast maturation and decreases collagen osteoblasts in the bone matrix.

Whereas in the day seven treatment group where hADMSCs and chitosan scaffold were added the serum calcium level had the highest increase compared to the negative control group, the control group was positive because hADMSCs were able to increase TGF-β levels so that the proliferation of undifferentiated mesenchymal stem cells increased osteoblast and chondroblast cells. In addition, hADMSCs also increase the production of TGF-β1 which increases osteoblast differentiation and reduces the ability of osteoblasts to osteoclast secretion by releasing OPG to inhibit osteoclast function for bone resorption so that bone mineralization processes can increase.[5] Thus the serum calcium level on the seventh day of the treatment group increased.[30,31]

The day fourteen of the serum calcium level increased in both the negative control group, the positive control group, and the treatment group compared to the day seven. This is because the fourteenth day is the most active phase of osteogenesis which will then continue until the day twenty one. In animals, the fourteenth day is the peak of hard callus formation.[18] Hard callus is a rigid and stable structure but does not yet have perfect biomechanical abilities like normal bones. Therefore on the day fourteen there is hard callus resorption by osteoclasts and the formation of woven bone by osteoblasts through a bone remodeling mechanism.[18] In this phase there is an increase in TNF-α, TNF-α indirectly inactivated osteoclasts through the production of IL-1 and IL-6 which will then encourage the occurrence of osteoclastogenesis.[1] The activity of osteoclastogenesis will result in increased extracellular calcium, therefore the serum calcium level in the blood is higher.[34]

Through this study, it can be proven that the application of Human Adiposed Derived Mesenchymal Stem Cells (hADMSCs) and chitosan scaffold can cause significant changes in calcium levels in blood serum both on day one, three, seven, and fourteen. This indicates that this vivo application of Human Adiposed Derived Mesenchymal Stem Cell (hADMSC) and chitosan scaffold can lead to an osteoinduction process.

Conclusions

The application of hADMSCs and chitosan scaffold caused a significant change in serum calcium levels on the day one, three, seven, and fourteen which mean that the combination of hADMSC and chitosan scaffold could trigger osteoinduction.

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Declaration of Interest

The authors report no conflict of interest.

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