Universidad Europea

TRABAJO DE FIN DE GRADO

Grado en Odontología

APLICACIÓN DE LAS CELULAS MADRES EN EL TRATAMIENTO DE LA ENFERMEDAD PERIODONTAL: SITUACIÓN ACTUAL Y PERSPECTIVAS DE FUTURO

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Índice abreviaciones

ADSCs: Células madre derivadas del tejido adiposo *(adipose-derived stem cells)*

BMMSCs: Células madre de la medula ósea *(bone marrow mesenchymal stem cells)*

β-TCP**:** beta-fosfato tricalcico

BIC: Contacto hueso-implante

CAL: Perdida de inserción clínica interproximal

DFSCs: Células madre del folículo dental *(dental Follicle stem cells)*

DPSCs: Células madre de la pulpa dental *(dental pulp stem cells)*

EDM: Proteínas derivadas de la matriz del esmalte

ESCs: Células madre embrionarias *(embryonic stem cells)*

GBR: Regeneración ósea guiada

GFs: Factores de crecimiento

GMSCs: Células madre derivadas del tejido gingival (gingival tissue derived stem cells)

HA: Hidroxiapatita porosa

HTA: Hidroxiapatita

IGF: Factor de crecimiento similar a insulina

iPSCs: Células madre pluripotenciales inducidas *(induced pluripotent stem cells)*

LAC: unión amelo-cementaría o cemento-adamantina

LPD: ligamento periodontal

PDLSCs: Células madre del ligamento periodontal *(periodontal ligament stem cells)*

PRP: Plasma rico en plaquetas

PVP: colágeno-polivinilpirrolidona liofilizada

SCAPs: Células madre de la papila apical *(stem cells from di apical papilla)*

SHEDs: Células madre de dientes deciduos humanos exfoliados *(stem cells from human exfoliated deciduos teeth)*

TGF- β: Factor de crecimiento transformante-beta

Resumen

Introducción: La enfermedad periodontal afecta a un alto porcentaje de la población, perjudicando la calidad de vida de los pacientes. En las últimas décadas se ha investigado mucho sobre la odontología regenerativa, en particular modo sobre las células madre presentes en nuestro cuerpo. Las MSCs parecen tener poderes proliferativos increíbles siendo capaces de dar vida a nuevos linajes de células más especializadas para formar nuevos tejidos. Muchos estudios han investigado sobre nuevos protocolos basados en células madre para la regeneración de los tejidos periodontales destruidos.

Objetivos: El objetivo principal del estudio ha sido establecer la eficacia de los tratamientos periodontales basados en células madre.

Materiales y métodos: Para la búsqueda bibliográfica se han utilizado Pubmed, Medline, Cochrane y la base de datos de la biblioteca CRAI de la Universidad Europea. Incluyendo artículos de revisiones bibliográficas y ensayos clínicos desde el 2006 hasta el 2020. Se ha llevado a cabo un estudio comparativo de las experiencias de los odontólogos en España, Inglaterra, Francia y Italia. Para esto se ha diseñado un breve cuestionario en Ingles, Español y Italiano.

Discusión: Las nueva clasificación de la periodontitis ha aportado mayor claridad para su diagnostico. Se han analizado todas las propiedades de las células madre de origen dental y no dental y los tejidos de donde se obtienen. Se ha llevado a cabo una revisión de ensayos clínicos que han reportado resultados significativos en tratamientos de regeneración periodontal en modelos animales y en humanos.

Conclusiones: Los resultados de los estudios en las terapias de regeneración han sido muy positivos, sin embargo, falta investigación para tener protocolos más seguros y predecibles para poder ser aplicados en la practica clínica.

Palabras claves: regeneración periodontal, células madre, ingeniería tisular, enfermedad periodontal, regeneración ósea, células mesénquimales.

Abstract

Introduction: Periodontal disease affects a high percentage of the population, damaging the quality of life of patients. In the last decades, much research has been done on regenerative dentistry, in particular on the stem cells present in our body. MSCs appear to have incredible proliferative powers, being able to give life to new, more specialized cell lines to form new tissues. Many studies have investigated new stem cell-based protocols for the regeneration of destroyed periodontal tissues.

Objectives: The main objective of the study has been to establish the efficacy of stem cell-based periodontal treatments.

Materials and methods: Pubmed, Medline, Cochrane and the database of the CRAI library of the Universidad Europea were used for the bibliographic search. Including articles from bibliographic reviews and clinical trials from 2006 to 2020. A comparative study of the experiences of dentists in Spain, England, France and Italy has been carried out. For this, a short questionnaire has been designed in English, Spanish and Italian.

Discussion: The new classification of periodontitis has provided greater clarity for its diagnosis. All the properties of stem cells of dental and non-dental origin and the tissues from which they are obtained have been analyzed. A review of clinical trials has been carried out that have reported significant results in periodontal regeneration treatments in animal models and in humans.

Conclusions: The results of the studies on regeneration therapies have been very positive, however, research is lacking to have safer and more predictable protocols to be applied in clinical practice.

Key words: periodontal regeneration, stem cells, tissue engineering, periodontal disease, bone regeneration, mesenchymal cells.

INDICE

1. INTRODUCCIÓN

La enfermedad periodontal, es una patología crónica inflamatoria, de origen multifactorial. [(1)]. Está caracterizada por una progresiva destrucción del periodonto, conjunto de tejidos altamente especializados que soportan y rodean el diente. Estos tejidos son: ligamento periodontal, encía, hueso alveolar propio y cemento[(2)]. La patogenia de esta enfermedad comprende un conjunto de factores que interactúan entre si, como la capacidad intrínseca del huésped de responder a la colonización microbiana de la inserción periodontal (una de las bacterias más estudiadas involucrada en el desarrollo de la periodontitis es la *Porphyromona Gingivalis)*, y factores modificables como hábitos de higiene oral, técnicas de cepillado, algunos alimentos y tabaquismo[(3)(4)]. La evidencia científica además ha encontrado una correlación entre la enfermedad crónica periodontal y enfermedades sistémicas como patologías pulmonares, cardiovasculares y diabetes [(4)]. Por eso, es muy importante que los pacientes se motiven a tener un estilo de vida saludable bajo varios aspectos, con el apoyo del personal sanitario y de las terapias en el gabinete dental[(2)].Tanto la gingivitis como la periodontitis son enfermedades que afligen a un alto porcentaje de la población y comprometen, de forma importante, la calidad de vida de los pacientes. El objetivo ultimo de los tratamientos periodontales es intentar restaurar la funcionalidad y la arquitectura de todos los componentes del periodonto que se han destruido durante los procesos patológicos[(3)]. Los tratamientos periodontales convencionales consisten en rellenar los defectos y remplazar los tejidos dentales destruidos con materiales naturales y sintéticos, no obstante, estos enfoques terapéuticos no garantizan una verdadera regeneración de la función y una arquitectura fisiológica de los tejidos[(5)(6)]. En los últimos años se han

desarrollado muchos estudios sobre la ingeniería de los tejidos y la medicina regenerativa que han abierto un camino para nuevos enfoques terapéuticos en este campo[7]. El objetivo de la ingeniería tisular es el de conseguir regeneración tisular mediante la combinación de biomateriales y mediatores biológicos y así obtener tejidos vivos restaurando los que se han perdido[5][6]. Las células mesénquimales se están revelando como elementos biológicos muy eficaces en el proceso de regeneración tisular y hablando de ingeniería tisular, se consideran la clave en las terapias de regeneración periodontal por su versatilidad en los procesos de diferenciación, teniendo la capacidad de modular la inflamación, característica típica de la periodontitis[5][6]. En este trabajo se analizará la efectividad de distintos tratamientos basados en células madre y utilizados en la enfermedad periodontal. A través el estudio de ensayos clínicos y de meta-análisis se describirá la situación actual y las posibles perspectivas futuras respecto a las nuevas técnicas basadas en el empleo de células madre y se intentará establecer su eficacia. Con ello, se pretende elaborar una guía para todo profesional que quiera tener más información sobre este tema, proporcionando datos sobre las nuevas técnicas diagnosticas de la enfermedad periodontal, describiendo las estructuras periodontales en su forma de salud y en su estado patológico y analizando todas las características de las células madre que se utilizan en las nuevas terapias y sus efectos sobre los tejidos a regenerar.

1.1 Periodonto

El conocimiento del origen de los tejidos involucrados en las estructuras dentales y como se relacionan entre sí es fundamental para entender su funcionamiento tanto en su estado de salud cuanto en su estado patológico. Es necesario, para poder desarrollar estrategias terapéuticas efectivas, sobre todo en los casos en los que nos encontramos frente a la perdida de la funcionalidad[4]. El periodonto está constituido por ligamento periodontal, encía, cemento y hueso alveolar propio. (Figura 1)

1.1.1 Ligamento periodontal:

El ligamento periodontal (LPD) se considera un tejido conectivo fibroso altamente especializado. Se sitúa en el llamado *espacio periodontal*, entre el cemento che cubre la raíz del diente y la lamina compacta periodontal del hueso alveolar propio. Este tejido está formado por células, fibras, sustancia fundamental amorfa, vasos y nervios. Entre otros tipos de células (formadoras, resortivas y defensivas) en la región del LPD podemos encontrar dos tipos de células madre: epiteliales y mesénquimales. Las células madre epiteliales (ESCs) se encuentran en las proximidades del ápice del diente y las células mesénquimales (MSCs) las encontramos en la zona perivascular. El LPD tiene la capacidad de actuar como un receptor sensorial, dando informaciones sobre la posición de la mandíbula durante la masticación y funcionar como reservorio para la homeostasis tisular, para la reparación y la regeneración [4][7].

1.1.2 Encía:

La encía se compone de un núcleo central de tejido conectivo, cubierto por varias capas de epitelio. Se mantiene fuertemente unida al hueso alveolar y se une al diente gracias a grupos

de fibras; colágenas (más numerosas e importantes), elásticas y reticulares. Los primeros dos grupos de fibras mencionados refuerzan la unión dentogingival. Dicha unión tiene la función de unir la encía al diente y está formada por tres componentes funcionales: surco o hendidura gingival, epitelio del surco y epitelio de unión (figura 1). El epitelio de unión forma un collar en la porción cervical del diente, alrededor de la corona clínica y tiene una fundamental importancia biológica a la hora de sellar los tejidos periodontales del medio oral y de mantener su integridad[(4)]. Su aspecto es triangular, teniendo su base al fondo del surco o hendidura gingival y su vértice en la unión cemento adamantina (LAC). El tejido conectivo subyacente al epitelio de unión tiene características diferentes con respecto al tejido conectivo que soporta el epitelio del surco. Presenta una alta vascularización y un alto numero de células inflamatorias (polimorfonucleares, linfocitos T y leucocitos) que migran a través del epitelio de unión hasta la hendidura gingival, extravasando, en ocasiones, en el fluido oral (Figura 2). Esta diferencia tiene un papel muy importante a la hora de entender la progresión de la enfermedad periodontal y los diferentes intentos de regeneración periodontal[(7)(8)].

1.1.3 Cemento:

El cemento es un tejido conectivo duro especializado avascular que cubre la raíz del diente y permite el anclaje de las fibras del ligamento periodontal. Se extiende desde la porción cervical del diente, en la unión con el esmalte (LAC) hasta el ápice[(7)]. Hay dos variedades de cemento, *celular y acelular*. El primer mencionado, empieza su formación antes que el diente erupcione hasta que entre en contacto con el antagonista. Se llama *cemento acelular* o *primario* y se encuentra desde el tercio cervical hasta los dos tercios de la raíz, se forma predominantemente for fibras altamente mineralizadas. El cemento *secundario* o *celular,* se

encuentra en el tercio apical y en las zonas interradiculares. Inicia a formarse cuando el diente entra en oclusión y continúa depositándose durante toda la vida, siendo un tejido de reparación para defectos de resorción y fracturas radiculares[8]. Es un mecanismo de compensación por el desgaste del diente por atrición durante toda la vida. El cemento celular presenta numerosos tipos de células, como los cementoblastos[4][7].

1.1.4 Hueso Alveolar:

El hueso alveolar, es la parte del hueso de los maxilares y de la mandíbula que contiene los alveolos. Presenta una estructura histológica igual a la del tejido óseo. Consiste en una vertiente correspondiente a la cara libre, constituida por tejido óseo compacto y revestida por periostio, se denomina *cortical compacta o periostica.* Una vertiente alveolar, *denominada cortical o compacta periodontica* también formada por tejido óseo duro. Esta vertiente está íntimamente relacionada con el LPD. Entre estas dos vertientes encontramos tejido óseo esponjoso o medular, en el cual encontramos embebidas las fibras de Sharpey que conectan el diente al hueso alveolar. A nivel de las crestas alveolares las dos vertientes se unen y es este punto no hay hueso esponjoso[8]. La presencia del hueso alveolar, que rodea el diente por toda su circunferencia, hace que este sea anatómicamente y funcionalmente separado del LPD. La organización de los procesos alveolares es un ejemplo muy importante de la relación entre estructura y función en el complejo periodontal [4].

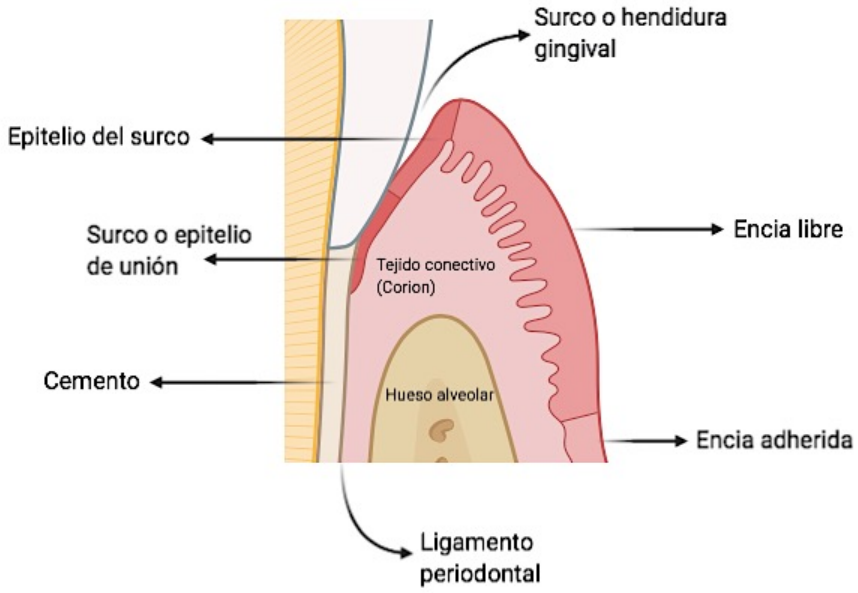

Figura 1

Corte sagital en sentido antero-posterior. Histología del periodonto[4] (Imagen elaborada por el autora partir de los datos proporcionados por Nanci et al.)

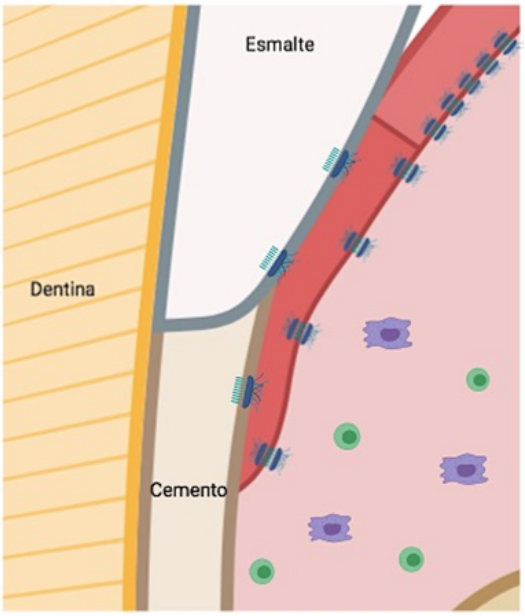

Figura 2

Desmosomas en la unión entre epitelio de unión y el tejido conectivo subyacente con presencias de células inflamatorias. Hemidesmosomas en la unión entre epitelio de unión y esmalte/cemento. Presencias de numerosos desmosomas en la unión entre epitelio del surco y tejido conectivo subyacente, donde no hay presencia de células inflamatorias[4] (Imagen elaborada por el autor a partir de datos proporcionados por Nanci et al.)

1.2 Enfermedad periodontal

La periodontitis es una enfermedad crónica multifactorial y inflamatoria, asociada a una biopelicula de placa disbiotica y caracterizada por una progresiva perdida de los tejidos de soporte de los dientes. La inflamación, típica de la periodontitis lleva a una perdida de inserción y la formación del biofilm bacteriano lleva a una inflamación gingival[(1)]. La enfermedad periodontal se caracteriza por tres factores principales:

1. Pérdida de los tejidos de soporte, que se manifiesta con la pérdida de inserción epitelial y con pérdida de hueso visible radiográficamente.
2. Presencia de bolsas periodontales.
3. Sangrado gingival.

Según la evidencia científica existen numerosos factores que influyen en la enfermedad periodontal, como el tabaco y la genética, y que existen también numerosas respuestas inmunoinflamatorias. Esto, hace que cada paciente desarrolle un tipo de respuesta diferente concorde a sus características y a sus hábitos[(1)]. Ya que el cuidado propio de los pacientes en el control de la placa es la base del tratamiento periodontal y los tiempos de los tratamientos periodontales generalmente son largos, la comunicación con el paciente es una de las claves para tener éxito en estos tratamientos[(2)]. El desequilibrio entre las especies bacterianas orales patógenas y las beneficiosas se ha demostrado ser un factor de inicio en la enfermedad periodontal. Normalmente esta disbiosis esta influenciada por una mala higiene por parte del paciente, que favorece el acumulo de bacterias como *Agregatibacter actinomycetemcomitans* y especialmente de bacterias del complejo rojo: *Porphyromona gingivalis, Tannerella forsythia* y *Treponema denticola*[(9)(6)]. Las bacterias son esenciales para

el desarrollo de la enfermedad, el hecho que progrese en formas distintas en diferentes individuos sugiere una etiología multifactorial de la periodontitis[4]. Cuando la integridad del epitelio de unión esta comprometida, las bacterias patógenas pueden acceder a las estructuras periodontales subyacentes. La presencia de estas bacterias activa la respuesta inflamatoria directa e indirecta, activando la respuesta inmune del huésped. Esta inflamación localizada, hace que se provoque una desintegración del tejido conectivo subyacente al epitelio de unión. El primer cambio visible durante el inicio del proceso patológico es la migración del epitelio de unión a lo largo de la superficie radicular, creando lo que se conoce clínicamente como *bolsa periodontal* (Figura 3). Esta falta de sellado y el aumento de superficie expuesta del diente permite el deposito de una mayor cantidad de placa y bacterias, causando una mayor inflamación[7][4][10].

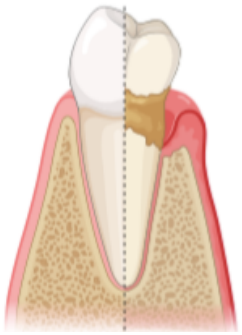

Figura 3

Diente sano vs diente con periodontitis: presencia de calculo, perdida de inserción y perdida de hueso alveolar. (Imagen elaborada por el autor a partir de las referencias [7,4,10])

1.2.1 Clasificación de la Enfermedad Periodontal

Las clasificación clásica de la enfermedad periodontal, publicada en 1999, ha proporcionado un marco de referencia y se ha utilizado ampliamente tanto en la practica clínica cuanto en la investigación científica. Sin embargo este sistema, sufre de defectos, de superposiciones y de falta de una clara diferenciación pato-biológica de las categorías[(1)]. Esto lleva a una dificultad de diagnostico. La Nueva Clasificación sacada por el World Workshop on Periodontal and Peri-implant Disease and Conditions ("the World Workshop") en 2017, revisionando la evidencia cientifica ha sacado 4 conclusiones[(1)(11)]:

1. No existe ninguna evidencia de una fisiopatología especifica que permita diferenciar los casos de periodontitis "agresiva" o "crónica" y proporcione una guida para diferentes tipos de tratamientos.
2. Hay poca evidencia que demuestre que la periodontitis agresiva y la crónica sean diferentes enfermedades. Hay evidencia de que múltiples factores, y la interacción entre ellos, influencie los resultados clínicamente visibles a nivel individual.
3. Basándonos en la población, la media que presenta una progresión de periodontitis, observando todas las poblaciones mundiales, es muy consistente. Hay evidencia de que segmentos específicos de la población exhiben diferentes niveles de progresión de la enfermedad.
4. Un sistema de clasificación basado solo en la severidad de la enfermedad toma el riesgo de no tener en cuenta factores importantes en la enfermedad individual, incluyendo la complejidad (que tiene influencia en el enfoque terapéutico) y los factores de riesgo (que influencian los resultados de la enfermedad).

Gracias a estas conclusiones y después de un largo debate, se ha creado un sistema multidimensional, basado en estadios y en grados para poder clasificar la enfermedad según características individuales especificas[12]. Los estadios describen la severidad de la enfermedad y la complejidad del manejo terapéuticos y los grados describen los niveles de progresión, las características biológicas y la evaluación de los factores de riesgos[1] (tablas. 1-2). Las formas de la enfermedad que antes se conocían como "crónica" y "agresiva" ahora se describen bajo una misma categoría: "periodontitis". Se han identificado tres formas de periodontitis según la fisiopatología[1]:

- Periodontitis necrosante
- Periodontitis como manifestación directa de enfermedades sistémicas
- Periodontitis, que debe ser caracterizada adicionalmente aplicando un abordaje de clasificación mediante estadios y grados[11].

Se ha aceptado definir la periodontitis como una patología que presenta una destrucción de los tejidos periodontales debida a la presencia de inflamación. Se ha establecido, como valor máximo, una perdida de inserción clínica interproximal (CAL) de ≥ 2 mm o ≥ 3 mm en dos o más dientes no adyacentes[12]. Cuando se presenta una perdida de inserción, se debería indagar sobre su origen y evaluar la presencia de factores locales como pueden ser: lesiones endo-periodontales, fracturas radiculares verticales, caries, octavos impactados [1].

		ESTADIO I	ESTADIO II	ESTADIO III	ESTADIO IV
Gravedad	**CAL interdental en el sitio de mayor pérdida**	1-2 mm	3-4 mm	≥ 5mm	≥ 5mm
	Pérdida ósea radiográfica	Tercio coronal (< 15 %)	Tercio coronal (15%-33%)	Se extiende hasta la tercio medio o apical de la raíz	Se extiende hasta la tercio medio o apical de la raíz
	Pérdida dentaria	NO	NO	≤ 4 de elementos perdidos debido a periodontitis	≥ 5 elementos perdidos debido a periodontitis
Complejidad	**Local**	Profundidad de sondaje máx. ≤4 mm Pérdida ósea horizontal	Profundidad de sondaje máx. ≤5mm Pérdida ósea horizontal	Profundidad de sondaje ≥ 6mm Pérdida ósea vertical ≥ 3mm Lesión de furca grado II o III Moderado defecto de la cresta	Profundidad de sondaje ≥ 6mm Necesidad de rehabilitación completa debido a: disfunciones masticatorias, trauma oclusal secundario (movilidad grado ≥ 2), defecto importante de la cresta ósea, migración dentaria , abanicamiento de los dientes y colapso de la mordida, 10 parejas de dientes en oclusión.

Tabla 1

Tabla elaborada a partir de la información de las referencias [11-12]; valoración de la enfermedad periodontal en estadios (I,II,III,IV), según criterios de gravedad y complejidad.

		Grado A	Grado B	Grado C
Evidencia directa	Rx o evaluación periodontal en los 5 años previos	No hay evidencia de perdida de hueso o inserción	< de 2mm de pérdida	Pérdida ≥ 2 mm
Evidencia Indirecta	Pérdida ósea	< 0,25 mm	0,25-1 mm	> 1 mm
	Aspecto	Bajos niveles de destrucción y grandes depósitos de *biofilm*	La destrucción es proporcional a los niveles de *biofilm*	Grado de destrucción alto respecto a los niveles de *biofilm*. Este cuadro clínico sugiere un patrón de patologías de progresión rápida y/o patologías de aparición temprana.
Factores que influyen	Tabaco	Paciente no fumador	< de 10 cigarrillos al día	≥ de 10 cigarrillos al día
	Diabetes	Paciente normal con o sin diabetes	HbA1c < de 7 con diabetes	HbA1c > de 7 con diabetes

Tabla 2

Tabla elaborada a partir de la información de las referencias [11-12]; valoración de la enfermedad periodontal por grados (A,B,C) según criterios de evidencia directa y indirecta y según factores que pueden influir.

Figura 4

Representación fotográfica de los estadios de la enfermedad periodontal[(1)].

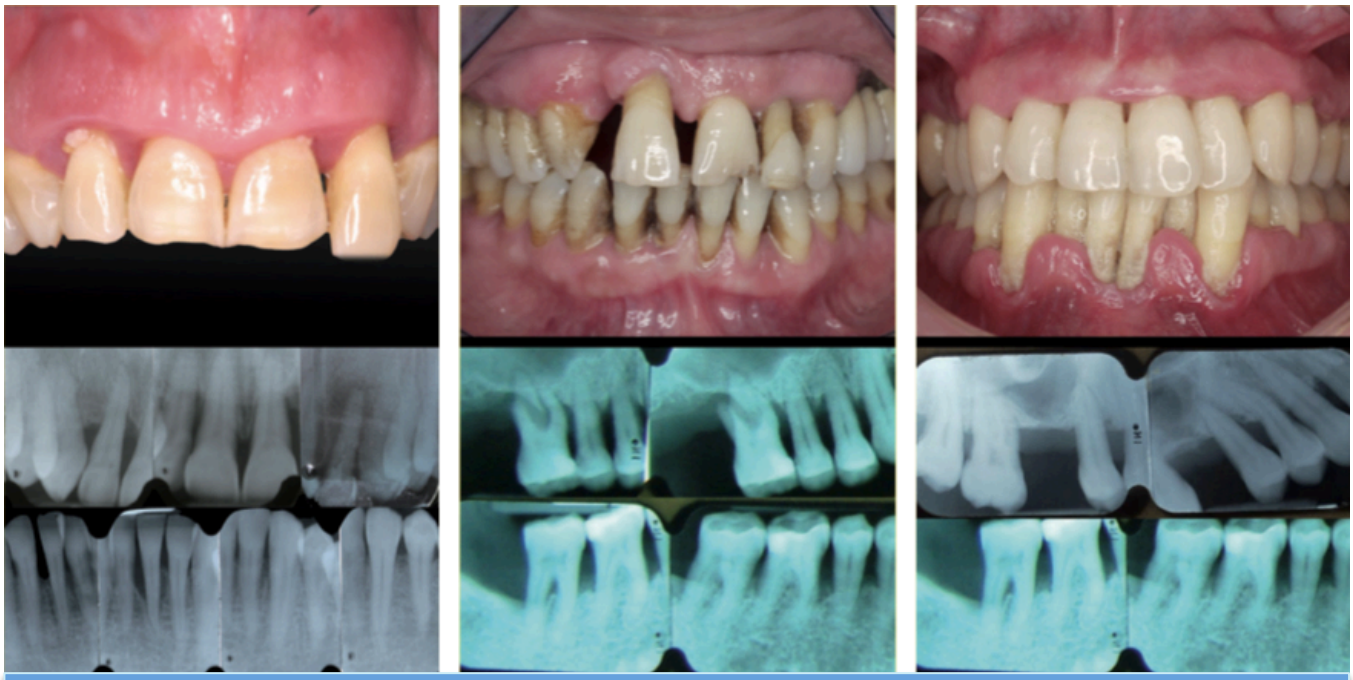

Figura 5

Imágenes fotográficas y radiográficas de los grados de la periodontitis [(1)].

2. OBJETIVOS

Para la realización de este trabajo se han planteado 1 objetivo principal y 3 secundarios;

Objetivo principal:

1. Analizar la efectividad de distintos tratamientos basados en células madre, para obtener una idea más clara sobre implantación, éxito y posibilidades de estas nuevas terapias. Esta análisis se obtendrá gracias a la comparación de estudios clínicos.

Objetivos secundarios:

2. Describir las células madre, los tejidos de obtención de estas últimas para aplicarlas en las terapias de regeneración periodontal.

3. A través los resultados reportados en los cuestionarios, reflejar sobre los actuales conocimientos y opiniones de los odontólogos.

4. Discutir las posibles aplicaciones futuras de las terapias regenerativas basadas en células madre.

3. MATERIALES Y MÉTODOS

Para la búsqueda de los artículos científicos se han utilizado como base de datos: Pubmed, Medline y Cochrane. Se han utilizado palabras clave como: regeneración periodontal, células madre, ingeniería tisular, enfermedad periodontal, regeneración ósea, células mesénquimales. Los libros y las revistas se han obtenido gracias a la base de datos digital en la Biblioteca CRAI Dulce Chacón de la Universidad Europea. Se han utilizado criterios de inclusión y exclusión por año de publicación y relevancia científica. Se han incluidos aquellos artículos que se han considerado de mayor relevancia en forma de revisiones bibliográficas y sobre todo de ensayos clínicos y de estudios de meta-análisis de revistas de impacto. Se han incluido estudios en un rango de publicación desde el año 2006 hasta 2020, excluyendo artículos anteriores. Se han excluidos estudios en idiomas diferentes de Inglés y Español. Para la realización del trabajo se han seleccionado 48 artículos. Las imágenes de producción propia se han obtenido utilizando el programa de diseño grafico Biorender obtenido gracias a Biorender.com. Se ha llevado a cabo un estudio comparativo de las opiniones y experiencias de los odontólogos sobre el uso de tratamientos basados en células madre. Para ello se ha diseñado un cuestionario corto que se incluye como anexos 1,2,3. El cuestionario incluye un consentimiento informado preliminar y se ha realizado en tres idiomas (Inglés, Español e Italiano) para que pueda ser leído, comprendido y llevado a cabo por profesionales de distintos países. La información de campo ha sido recogida en formato presencial y en formato online. Para los resultados de las encuestas, los gráficos se han obtenido gracias al programa de recolección de los datos Google Forms.

4. RESULTADOS:

Figura 6

El 100% de los odontólogos han aceptado las condiciones del consentimiento informado.

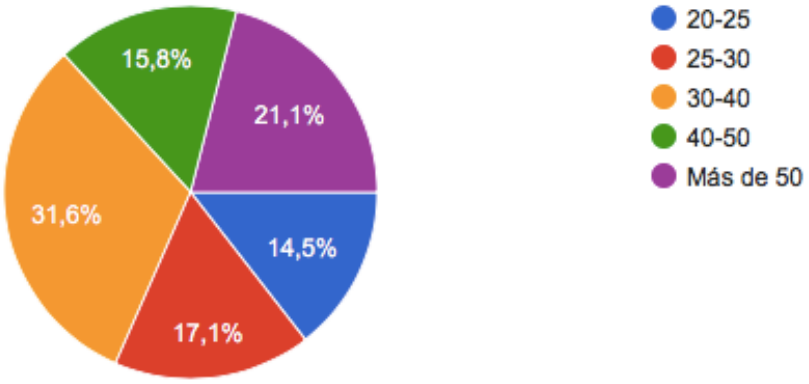

Figura 7

El diagrama de sectores representa la edad de los encuestados. Se recogieron un total de 76 respuestas.

Figura 8

El diagrama de sectores representa los conocimientos de los encuestados inherentes a los tratamientos sobre células madre. Se recogieron un total de 76 respuestas. El 35,5% ha respuesto "*no, nunca me he informado pero el tema me interesa y lo haré en futuro*".

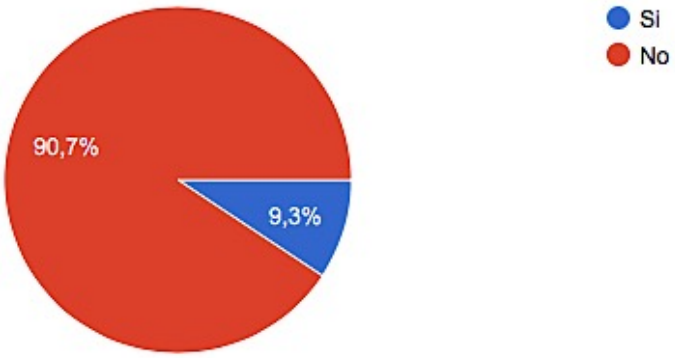

Figura 9

El diagrama de sectores representa el total de los encuestados que han participado en estudios sobre células madre en el tratamiento de la enfermedad periodontal o que han aplicado este tratamiento en pacientes. Se recogieron un total de 76 respuestas. El 9,3% ha respuesto afirmativamente.

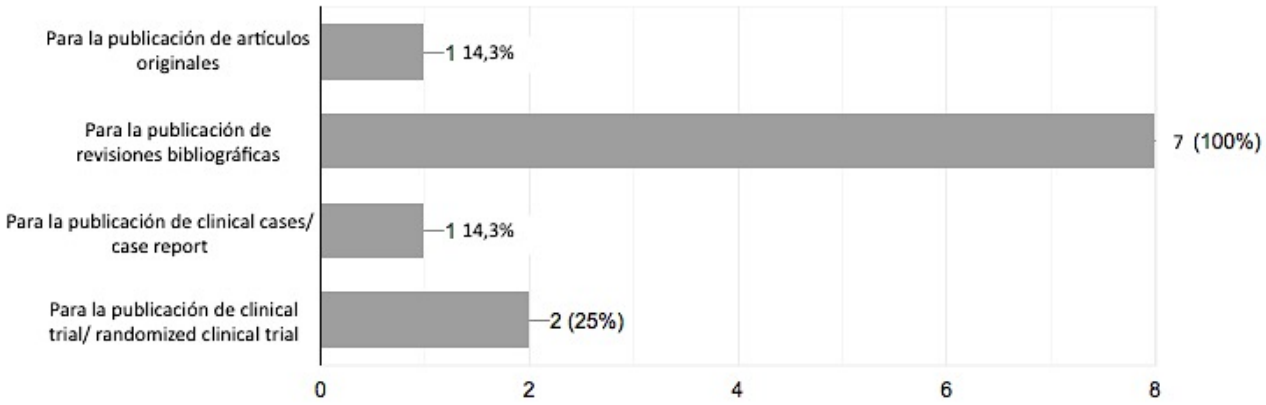

Figura 11

El histograma representa la distribución de los estudios cursados por los participantes. Se recogieron un total de 7 respuestas.

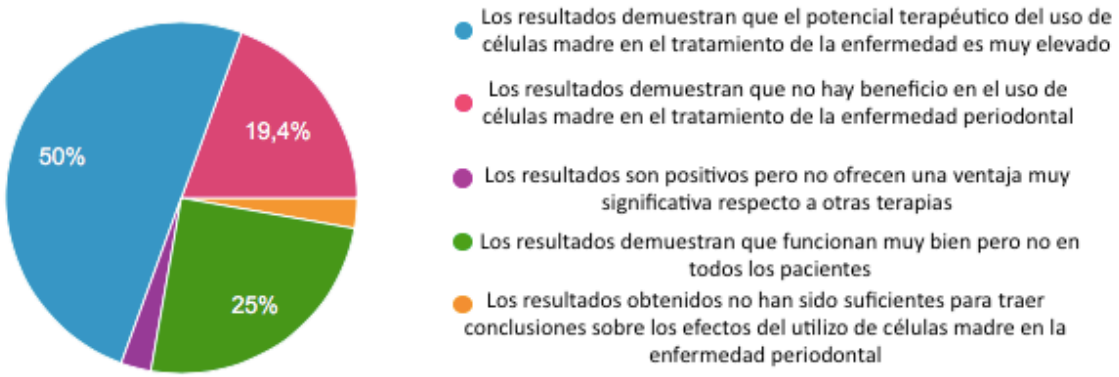

Figura 10

El diagrama de sectores recoge la opinión e impresiones de los participantes sobre los estudios cursados o experiencias clínicas. Se obtuvieron un total de 36 respuestas. El 50% de los participantes opina que "*los resultados demuestran que el potencial terapéutico del uso de células madre en el tratamiento de la enfermedad periodontal es muy elevado*"

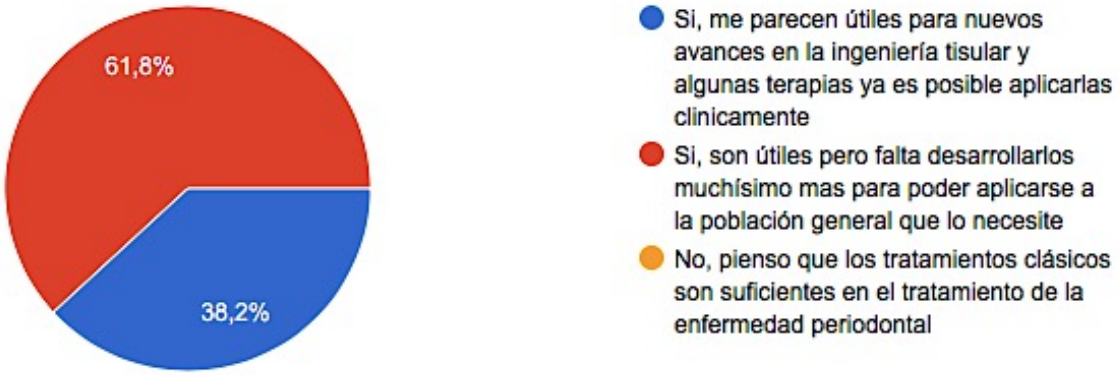

Figura 12

Más concretamente en este diagrama de sectores se muestra la distribución de las opiniones sobre el potencial terapéutico de las células madre y sobre su investigación. Se han obtenido un total de 76 respuestas. El 61,8% opina *" Si, me parecen muy útiles para nuevos avances en la ingeniería tisular y algunas terapias ya es posible aplicarlas clínicamente".*

5. DISCUSIÓN

5.1 Tratamientos modernos y sus limitaciones

Regenerar los tejidos perdidos es de importancia principal no solo en procesos como la periodontitis sino también en otros procesos que sufren pérdida de hueso como tumores, fracturas y defectos óseos[13]. El objetivo último de las terapias periodontales es la regeneración del hueso alveolar, del cemento y del ligamento periodontal[14]. Está probado científicamente y ampliamente aceptado que el manejo de la enfermedad periodontal deba empezar con un protocolo de control de la inflamación, que es absolutamente indispensable para reducir la carga bacteriana y para crear un medio apropiado para tratamientos regenerativos. Normalmente el manejo de la inflamación se controla con tratamientos de raspado y alisado radicular, profilaxis, y un protocolo de higiene oral en casa estricto. Solo cuando se obtiene un control optimo de la inflamación y un control periódico de la periodontitis con índices de progresión, es posible plantear hacer tratamientos regenerativos[15]. Aunque el periodonto exhibe cierta capacidad regenerativa intrínseca, no se puede esperar una restitución natural *ad integrum* sin la intervención de terapias regenerativas externas[16]. El primer tratamiento periodontal de regeneración consiste en el uso de biomateriales como injertos óseos, materiales bioactivos y membranas para realizar lo que se conoce como Regeneración Ósea Guiada, en Inglés *Guided Bone Regeneration* (GBR). Dependiendo del origen, los injertos óseos pueden ser autógenos, alógenicos, aloplásticos y xenogénicos[7]. El termino “materiales bioactivos” se utiliza para hablar de sustancias que se utilizan para estimular el crecimiento del hueso. Éstas sustancias normalmente son los denominados “factores de crecimiento” (GFs), que estimulan y reclutan células madre para lograr regeneración. Las fuentes de factores de crecimientos

mayormente utilizados para la ingeniería periodontal de los defectos óseos son: plasma rico en plaquetas (PRP), proteínas derivadas de la matriz del esmalte (EMD), proteínas morfogenéticas del hueso (BMPs)[7]. El PRP ha sido definido por primera vez en 2007 como una preparación de plaquetas presente en un volumen pequeño de plasma sanguíneo conteniente una gran cantidad de GFs. De hecho, en PRP hay 15 variedades de GFs, las principales son: factor de crecimiento derivado de plaquetas (PDGF), factor de crecimiento similar a la insulina (IGF) y factor de crecimiento transformante beta (TGF- β). Sin embargo, la duda que rige si el PRP es clínicamente eficaz o no se ve agravada por el conocimiento inadecuado sobre la biodisponibilidad de los GF derivados de PRP. Los beneficios que conlleva la aplicación del PRP en la regeneración ósea implican su disponibilidad, facilidad de aislamiento, buenas propiedades de manipulación y almacenamiento y su aplicación en el campo de la ingeniería del tejido óseo. Además el PRP es autólogo, lo que elimina el riesgo de enfermedad, transmisión y rechazo inmunológico[13]. De hecho, el problema asociado con estos tipos de terapias es cuando se utilizan biomateriales homo-heterólogos, pudiendo el paciente presentar reacciones de rechazo. En un estudio publicado en 2018 por Quintessence, se ha reportado que las complicaciones de los tejidos blandos después de la regeneración ósea guiada (GBR) ocurren en el 16,8% de los casos[17]. El tratamiento de los tejidos blandos sigue siendo el factor clave para evitar complicaciones de los tejidos blandos y, por tanto, para aumentar el éxito de la terapia regenerativa ósea. Además la evidencia cientifica demuestra que, los tratamientos convencionales de regeneración periodontal dependen mucho del numero de paredes óseas residuales, que proveen un soporte mecánico para el material de substitución ósea un adecuado aporte vascular[18]. Por lo tanto, la GBR se considera como tratamiento limitado y con relativa predictibilidad clínica [19][14][20].

5.2 Células Madre y Enfermedad Periodontal

5.2.1 Células madre en el cuerpo humano

Las células madre están presentes naturalmente en el cuerpo humano, en diferentes estadios de diferenciación. Muy a menudo se describen en presencia de las zonas peri-vasculares, esta localización anatómica permite a estas células su rápida movilización al sitio de la lesión [21]. Se caracterizan por su habilidad de renovación, junto con sus capacidades de diferenciación en múltiples linajes, permitiendo una compleja regeneración de los tejidos que lo necesiten. De hecho, se definen como clonogénicas, autorenovadoras y progenitoras, pudiendo generar una o más tipos de células. Sus funciones principales son el mantenimiento de la homeostasis y la reparación de los tejidos dañados. Tienen varios niveles de potencia; totipotentes, pluripotentes, multipotentes y unipotentes: las totipotentes pueden originar cualquier célula de cualquier capa embrionaria, las pluripotentes, cualquier célula de determinada capa embrionaria. Estos dos primeros grupos pueden por tanto originar distintos tipos de tejidos. Las multipotentes pueden dar lugar a cualquier célula de un solo tipo de tejido y por último las unipotentes solo pueden diferenciarse en un único tipo celular (Figura 13). Las células madre pluripotentes incluyen las células madre embrionarias (ESCs) que derivan del estrato interno del blastocito en su estadio de desarrollo y las células madre pluripotentes inducidas o reprogramadas (iPSCs). Las iPSCs son células madre generadas artificialmente a partir de células que en principio no eran pluripotenciales. El proceso requiere la alteración de la exposición de una serie de genes, lo que les induce a regresar a un estadio anterior menos diferenciado, convirtiéndose en células madre de linaje especifico para que se puedan, a su vez, convertir en células diferenciadas de determinado tipo[22]. Las células madre adultas o post-natales (ASCs) se

consideran multipotentes e incluyen linajes de células madre hematopoyéticas, células madre epidérmicas, células madre limbares y células madre mesénquimales, entre las cuales se encuentran las células madre adiposas, células madre hepáticas y células madre de los tejidos orales (Figura 13). En efecto, los tejidos dentales y cráneo faciales se conocen como reservorios importantes de MSCs y los dentistas tienen un acceso relativamente fácil a estos [21].

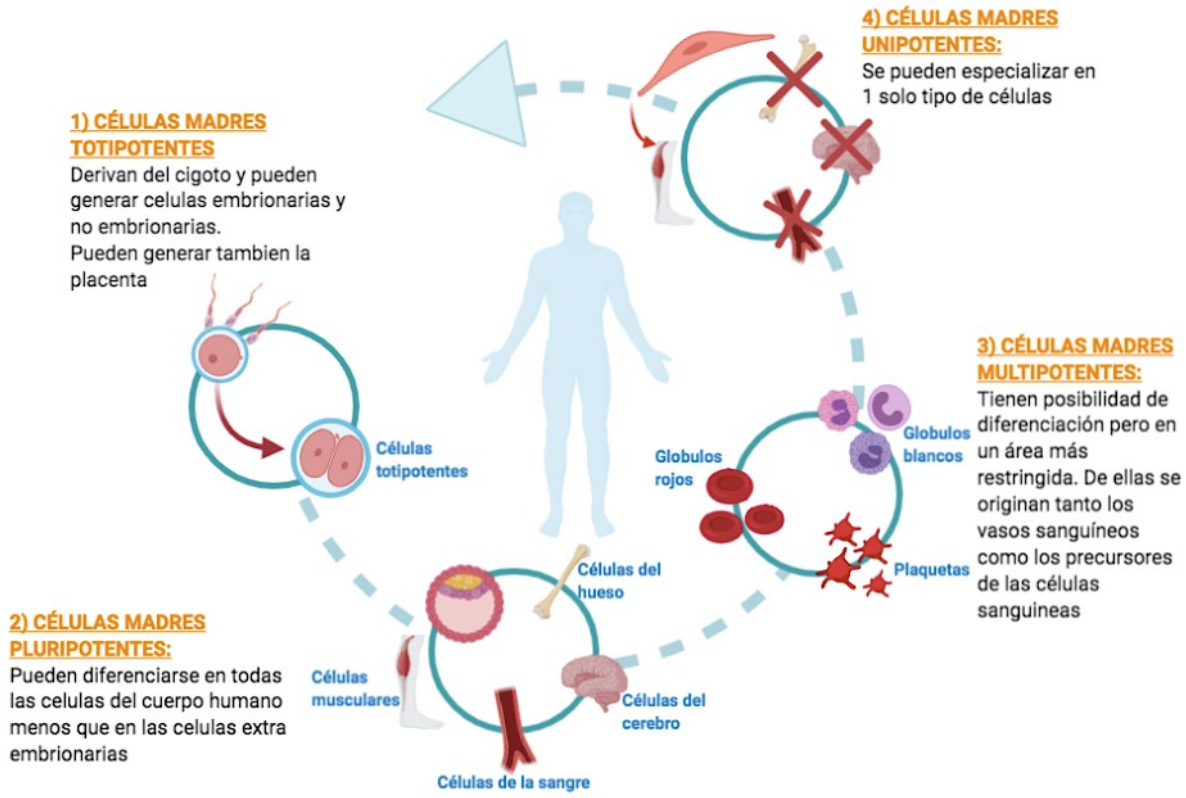

Figura 13

Células madre; desde las totipotentes hasta las unipotentes. (Imagen de elaboración del autor)

Las MSCs son células multipotentes con alto potencial de diferenciación clonogénica. Están presentes en el estroma adulto de la medula ósea. Tienen forma de huso y son similares a los fibroblastos. Además, las MSCs pueden diferenciarse en muchos tejidos de origen mesénquimal como hueso, cartílago, tendones, tejido adiposo, músculos y estroma de la medula. Las MSCs no solo están presentes en el estroma de la medula ósea, sino también es posible obtenerlas del tejido adiposo y de la sangre del cordón umbilical[(22)]. En las dos ultimas décadas la búsqueda de células similares a MSCs en tejidos específicos ha llevado al descubrimiento de variedades de células madre en casi todos los órganos y los tejidos de nuestro cuerpo (Tabla 4) incluyendo el descubrimiento de MSCs en tejidos orales/dentales [(22)(23)]. En los tejidos dentales se ha encontrado la presencia de numerosos tipos de células madre y se están estudiando sus potenciales y sus posibles aplicaciones en los tratamientos periodontales[(19)] (Tabla 3). Recientes investigaciones se han centrado en el estudio de las células madre aplicadas en la ingeniería tisular para la regeneración periodontal[(3)]. Las MSCs se consideran células muy adecuadas en el tratamiento periodontal, no solo por la capacidad de regenerar diferentes tipos de tejidos sino también por su potencial paracrino. De hecho, secretan importantes cantidades de factores de crecimiento, citoquinas anti-inflamatorias, como el TGF-β (factor de crecimiento transformante) e interleucinas (IL)–10 que juegan un papel muy importante en la inmunomodulación sistémica y local[(14)]. Se ha demostrado que las MSCs, en trasplantes alógenicos (o alostrasplante), en modelos porcinos con defectos óseos, no inducen rechazo inmunológico[(24)]. Las células madre mesénquimales utilizadas en la regeneración dental y periodontal son células madre dentales y no dentales (Tablas 3 y 4).

5.2.2 Células madre mesénquimales de origen dental

Recientes estudios han aislado MSCs desde varios tejidos dentales y orales. Se han aislado células madre de dientes deciduos exfoliados (SHEDs), con potencial osteo/odontogenico, condrogenico, adipogenico y neurogenico y se ha visto, además, que tienen una capacidad proliferativa mayor de las células madre derivadas del la medula ósea (BMMSCs) y de las células madre derivadas de la pulpa dental (DPSCs)[25]. Las DPSCs fueron las primeras células en ser aisladas de la pulpa de un adulto humano (Gronthos y cols. en el 2000). Éstas células demuestran un gran potencial de proliferación y de auto-renovación. En el mismo estudio, las DPSCs asociadas a matrices de hidroxiapatita y fosfato tricalcico (HTA/TCP) han producido una estructura muy similar a la dentina, con la pulpa dental en su interior y un linaje de odontoblastos, después de haber sido trasplantadas en ratones inmunodeprimidos [26]. Las PDLSCs fueron descubiertas por primera vez en 2004 gracias a Gronthos, Miura y cols. Los autores han demostrado que éstas células, *in vitro*, se pueden diferenciar en osteoblastos, células adiposas y células formadoras de colágeno[26]. Las PDLSCs se han empleado en estudios clínicos para observar la regeneración periodontal. Un ensayo clínico llevado a cabo por Akiyama y cols. en 2010 indica el potencial regenerativo de las PDLSCs en 3 pacientes con una enfermedad periodontal muy avanzada; 2 de estos pacientes han recuperado el tejido periodontal sano con una regeneración clínica razonable, la pérdida del grado de profundidad de sondaje del tercer paciente se redujo significativamente y se estabilizó la inserción periodontal[27]. La papila apical, solo se encuentra en dientes en crecimiento, antes que se complete el desarrollo de la raíz. Esta contribuye, de manera significativa, a la formación del diente. Las células madre que derivan de la papila apical (SCAPs) son células mesénquimales aisladas de la papila apical de dientes permanentes

inmaduros[26]. Se ha evidenciado que las SCAPs tiene un nivel de proliferación adecuado a la regeneración dental basada en células. Las SCAPs, además, se consideran más adecuadas con respecto a las DPSCs, por tener una mayor capacidad proliferativa y un buen potencial de mineralización. Las SCAPs parecen ser una fuente primaria de odontoblastos al formar la dentina radicular, mientras las DPSCs son la fuente probable de reemplazo de odontoblastos para formar dentina reparadora[26]. El folículo dental es una cápsula de tejido conectivo laxo que deriva del ecto-mesénquima y que envuelve el germen del diente en crecimiento. El folículo dental contiene células progenitoras que se pueden diferenciar en el células del ligamento periodontal, odontoblastos y cementoblastos. Las células del folículo dental (DFSCs) son las células madre más comúnmente extraídas de los alveolos de los terceros molares[26]. Después de haber sido trasplantadas en ratas, las DFSCs se han diferenciado en células del LPD, que secretando colágeno han interactuado con la superficie del hueso adyacente y con el cemento, generando después un tejido similar al LPD y al cemento[28]. Las células mesénquimales gingivales (GMSCs) se admiten como muy útiles debido a su enorme capacidad de regeneración, en cicatrización, clonogenicidad, propiedades inmunomoduladoras y propiedad de diferenciación multipotente como las otras MSCs[29]. GMSCs se consideran una población de células fácilmente accesibles ya que los tejidos gingivales se pueden obtener desde procedimientos odontológicos generales y tratadas como un desecho biomédico. De hecho el tejido gingival se puede obtener durante exodoncias o durante cirugías periodontales[30][31].

CELULAS MADRE DE ORIGEN DENTAL

ACRONIMO	TIPO DE CELULA MADRE	FORMACIÓN DE TEJIDO "DE NOVO"	APLICACIONES EN LA REGENERACIÓN DE DIENTE Y PERIODONTO
DFSCs	Células madre del folículo dental *(dental Follicle Stem Cells)*	Tejido similar al LPD y tejido similar al cemento	**Raíz del diente y regeneración del tejido periodontal**
DPSCs	Células madre de la pulpa dental *(dental pulp stem cells)*	Tejido similar a pulpa y dentina, tejido similar al hueso, cartílago, carácter angiogenico, tejido neuronal.	**Pulpa, dentina, raíz del diente y regeneración periodontal**
PDLSCs	Células madre del ligamento periodontal *(periodontal ligament stem cells)*	Tejido similar al cemento y tejido similar al LPD	**Regeneración periodontal**
SCAPs	Células madre de la papila apical *(stem cells from apical papilla)*	Tejido similar a pulpa y a dentina, carácter angiogenico.	**Pulpa, dentina y raíz del diente.**
SHEDs	Células madre de dientes deciduos exfoliados humanos *(stem cells from human exfoliated deciduous Teeth)*	Tejido similar a pulpa y dentina, tejido similar al hueso, carácter angiogenico, tejido nervioso.	**Pulpa, dentina, raíz del diente, regeneración periodontal.**
GMSCs	Células madre derivadas del tejido gingival *(gingival tissue derived stem cells)*	Tejido similar al LPD y tejido similar al cemento	**Raíz del diente y regeneración del tejido periodontal.**

Tabla 3

Tabla elaborada a partir de datos recogidos en las referencias [39]

5.2.3 Células madre mesénquimales de origen no dental

Las células madre mayormente utilizadas para la regeneración periodontal son las derivadas de la medula ósea (BMMSCs), las derivadas del tejido adiposo (ADSCs), las células embrionarias (ESCs) y las células pluripotenciales inducidas (iPSCs). Las BMMSCs han sido las primeras células mesénquimales en ser descubiertas y han presentado un potencial de diferenciación osteogénico, condrogénico, adipogénico y miogénico[(19)]. Para la regeneración periodontal y dental, como reportan los autores Ohazama, Modino, Miletich y Sharpe en un estudio del 2004, las BMMSCs pueden regular la expresión de genes odontogénicos y pueden así contribuir con la regeneración en sistemas mixtos con epitelio embrionico oral[(19)(32)]. Sin embargo, el procedimiento de obtención de estas células es bastante invasivo, acompañado de morbilidad y de malestar post-quirúrgico. Un criterio ideal de aplicación de células en medicina regenerativa incluye su facilidad de acceso y su asilamiento, conduciendo a la menor morbilidad para el paciente. Las células derivadas del tejido adiposo (ADSCs), son muy abundantes y se utilizan ampliamente en medicina regenerativa. En 2017 se ha publicado en el *Stem Cells Traslational Medicine* un estudio llevado a cabo en el University Hospital of Toulouse por los autores Lemaitre y cols.[(33)]. En este estudio se utilizan las ADSCs trasplantadas para tratar defectos óseos en roedores. En general los resultados conseguidos por los autores demuestran que las ADSCs pueden tener un papel muy significativo en la regeneración periodontal, no solo por inducir la regeneración del cemento, si no también, por primera vez, por inducir la organización de las fibras del LPD y del numero de vasos sanguíneos, así como células progenitoras y linajes de marcadores de células periodontales. Los autores concluyen que las ADSCs pueden ser un biomaterial de trasplante optimo para terapias periodontales[(19)(33)]. En 2011 ya se habían hechos estudios sobre ADSCs

y los autores Hung y cols. habían demostrado conseguir la regeneración de dentina, LPD y hueso alveolar, gracias al trasplante de ADSCs en los alveolos de conejos en laboratorio, revelando también un mayor potencial de estas últimas en comparación con las células madre derivadas de la pulpa dental (DPSCs)[34]. Las células madre embrionarias (ESCs), como ya se ha mencionado antes, son pluripotenciales y pueden diferenciarse en células odontogénicas y periodontogénicas en un medio cultivo o co-cultivadas con las células madre derivadas del LPD (PDLSCs) o también con células del epitelio embrionario oral[19]. Las células madre pluripotenciales inducidas (iPSCs) se descubrieron por primera vez en 2006 por parte de un estudio llevado a cabo por la Universidad de Kyoto. Durante este estudio se demostró la importancia y el poder clínico de estas células madre en la medicina regenerativa[35]. Las iPSCs se pueden diferenciar en los tejidos de las tres capas germinales: endodermo, mesodermo y ectodermo. Tienen, por lo tanto, la capacidad de regenerar los tejidos dentales y pueden ser generadas a partir de la reprogramación de células adultas especializadas. Se pueden generar también de células de tejidos dentales, que se consideran una fuente atractiva, debido a su accesibilidad y a su relativa simplicidad de consecución [19] [36]. Así mismo, para su utilizo en odontología, es preferible obtener iPSCs a partir de tejidos dentales. Las iPSCs se han generado con éxito a partir de células de la pulpa dental derivadas de dientes deciduos exfoliados (SHEDs), células madre de la papila apical (SCAPs) y células madre de la pulpa dental (DPSCs) (Yan et al. 2010) [37]; células madre inmaduras de la pulpa dental humana (Beltrao-Braga y cols. ;Yan y cols. y Dambrot y cols.)[37][38][39]; células de la pulpa dentaria de dientes deciduos perdidos naturalmente (Dambrot et al.)[39]; tejido gingival (Egusa et al.; Wada et al.)[31][40]; y células madre PDL (PDLSCs) (Wada et al. 2011) [40]. Durante una comparación realizada por Yan et al. en 2010 identificaron que la eficiencia de

reprogramación parecía ser mayor en los tejidos de origen dental que de las células de fibroblastos humanos [37] (Figura 14) .

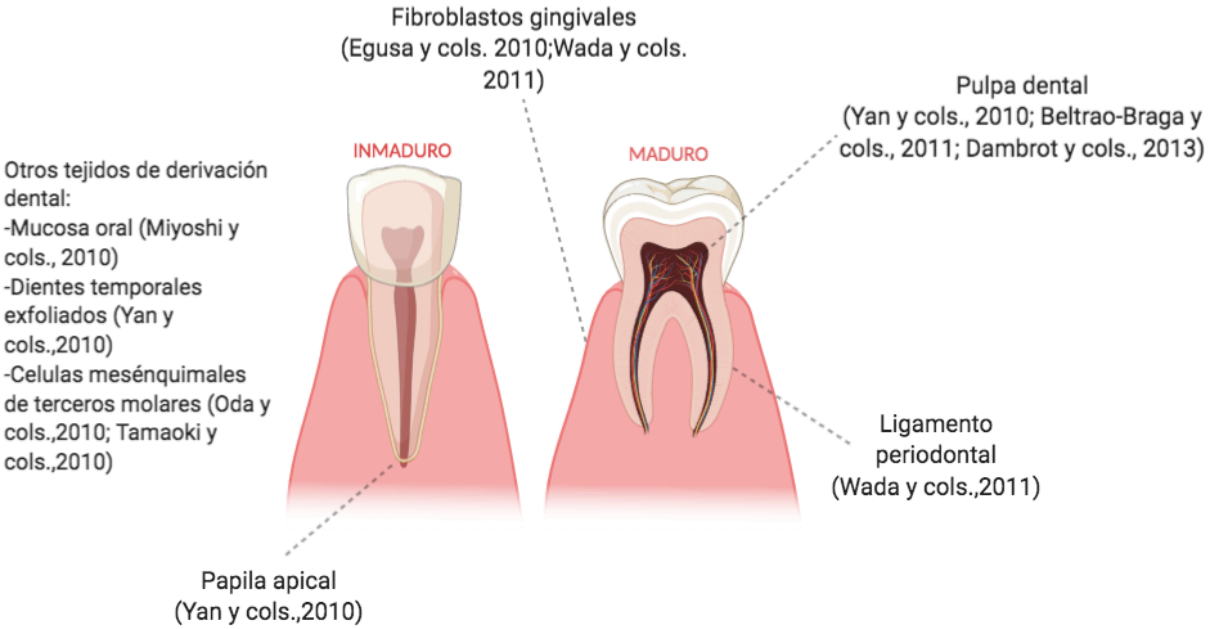

Figura 14

Tejidos dentales desde donde se han obtenido iPSCs. Imagen elaborada por el autor a partir de datos recogidos por las referencias [37]

CELULAS MADRE DE ORIGEN NO DENTAL

ACRÒNIMO	TIPO DE CELULA MADRE	FORMACIÓN DE TEJIDO "DE NOVO"	APLICACIONES EN LA REGENERACIÓN DE DIENTE Y PERIODONTO
BMMSCs	Células madre derivadas de la medula ósea *(bone marrow mesenchymal stem cells)*	Tejido similar al hueso, cartílago, musculo, tejido neuronal, tejido dentario.	**Regeneración de tejido periodontal y del diente entero**
ADSCs	Células madre derivadas del tejido adiposo *(adipose-derived stem cells)*	Tejido similar al hueso, carácter angiogenico, cartílago, tejido neuronal.	**Regeneración periodontal**
iPSCs	Células madre pluripotenciales inducidas *(induced pluripotent stem cells)*	Tejido similar al hueso, tejido miocardico, carácter angiogenico.	**Regeneración periodontal y del diente entero**
ESCs	Células madre embrionarias *(embryonic stem cells)*	Tejido similar al hueso, cartílago, tejido miocardico, carácter angiogenico.	**Regeneración periodontal y del diente entero.**

Tabla 4

Tabla de elaboración propia a partir de datos recogidos en la referencia[19]

5.2.4 Aplicaciones de células madre como tratamiento de la enfermedad periodontal

En 2016 se llevó a cabo el primer ensayo clínico (Katagiri y cols.) en humanos que demuestra el éxito de una regeneración alveolar a través el uso de BMMSCs. Para el estudio se han seleccionaron 8 pacientes a los cuales se les había indicado la necesidad de aumento óseo, incluyendo elevación de seno maxilar y regeneración ósea guiada y preservación alveolar. Estos pacientes, sufriendo de una importante atrofia ósea, tenían problemas de retención de las prótesis removibles y la colocación de implantes se consideró como posible solución. Los criterios de aplicación para estos procedimientos fueron presencia de menos de 5 mm de hueso residual desde el piso del seno maxilar hasta la cresta alveolar en los casos de elevación del seno y menos de 10 mm de hueso residual en los casos de GBR. Las células BMMSCs utilizadas fueron preparadas desde medios acondicionados a partir de medula ósea humana comercialmente disponible (Lonza inc. Walkersville, MD, USA). Todos los pacientes pasaron por un estricto control oral y un examen general. Durante la cirugía, las BMMSCs fueron disueltas en 5 ml de solución salina y como mediador utilizaron beta-fosfato-tricalcico. En los casos de defectos más pequeños de preservación alveolar como biomaterial utilizaron esponjas de Atelo-colágeno (un colágeno que puede ser implantado en pacientes de forma segura ya que es modificado para ser inmunogénico) y beta-fosfato tricalcico (β-TCP) (Figura 15). El estudio consiguió evaluar la fiabilidad clínica y la eficacia de las BMMSCs en ensayos clínicos humanos para la regeneración ósea. El todos los casos llevados a cabo no se encontraron hinchazones anormales o cicatrización tardías. El beta fosfato tricalcico se ha utilizado mucho tanto en lesiones ortopédicas cuanto el lesiones maxilofaciales y ha demostrado siempre optimas características osteoconductoras; sin embargo, es un material que se reabsorbe en un largo periodo de tiempo. En este estudio se demostró que el beta

fosfato tricalcico en combinación con BMMSCs promueve su reabsorción temprana y la substitución por hueso nuevo comparado con el beta fosfato tricalcico sin células mesénquimales. Sin embargo, siendo el grupo de pacientes un grupo pequeño, no se pudo mostrar y discutir en profundidad sobre los efectos radiográficos e histológicos. Los autores reportan en las conclusiones que se ha comenzado un ensayo clínico de la siguiente fase del estudio para evaluar la eficacia de BMMSCs en la regeneración ósea alveolar en un grupo con un mayor numero de pacientes [41]. En 2018 el *Journal of International Medical Research* ha reportado un exitoso caso clínico de injerto alógenico con células mesénquimales derivadas de la pulpa dental (DPSCs) en un paciente con enfermedad periodontal (Beatriz Hernandez-Monjaraz et al.)

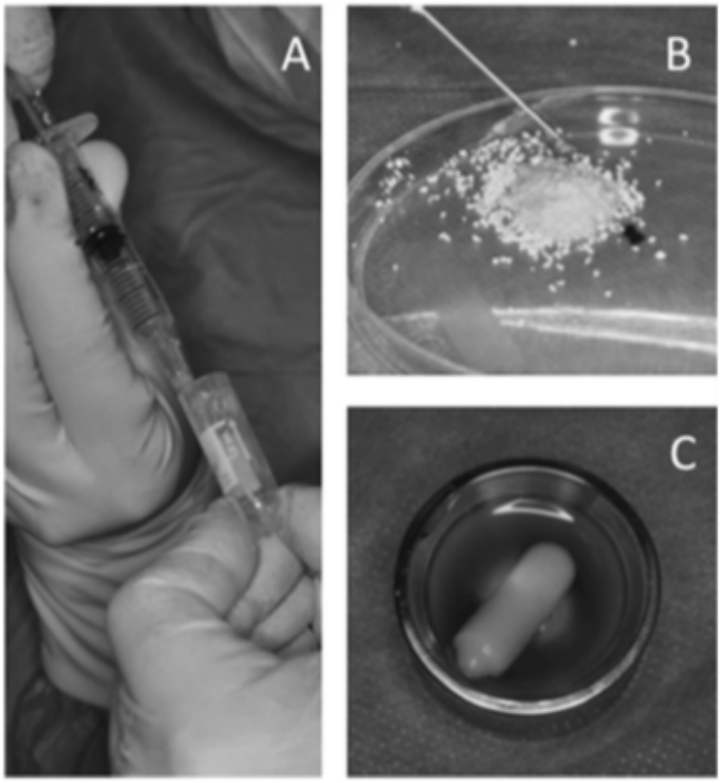

Figura 15

Preparación de BMMSCs para la implantación con diferentes mediadores. A: BMMSCs liofilizadas y disueltas en solución salina durante la cirugía. B: BMMSCs mezcladas con beta-fosfato tricalcico. C: esponja de Atelo-colágeno en una solución de BMMSCs[41]

Las DPSCs fueron separadas desde la pulpa cameral de un diente de un donador de 7 años de edad. Las células fueron procesadas vía enzimática y centrifugadas. Como biomaterial mediador se empleó una esponja de colágeno-polivinilpirrolidona liofilizada (PVP) y las DPSCs fueron diluidas en 250 µl de solución salina tamponada con fosfato. El acceso se consiguió tramite una cirugía a colgajo en la zona de los premolares en el tercer cuadrante (34-35). Durante el *follow up* en 3 y 6 meses el paciente no mostró ningún signo de rechazo y se verificó una disminución en la movilidad de los dientes, disminución de las bolsas periodontales y de los defectos óseos. Se observó, además, un aumento en la densidad mineral del hueso en la zona del injerto. Los autores concluyeron que los resultados de este caso clínico sugieren que los tratamientos con DPSCs promueven la regeneración periodontal, aunque más investigaciones futuras deben incluir ensayos clínicos aleatorizados para verificar los resultados [42] (Figuras 16-17).

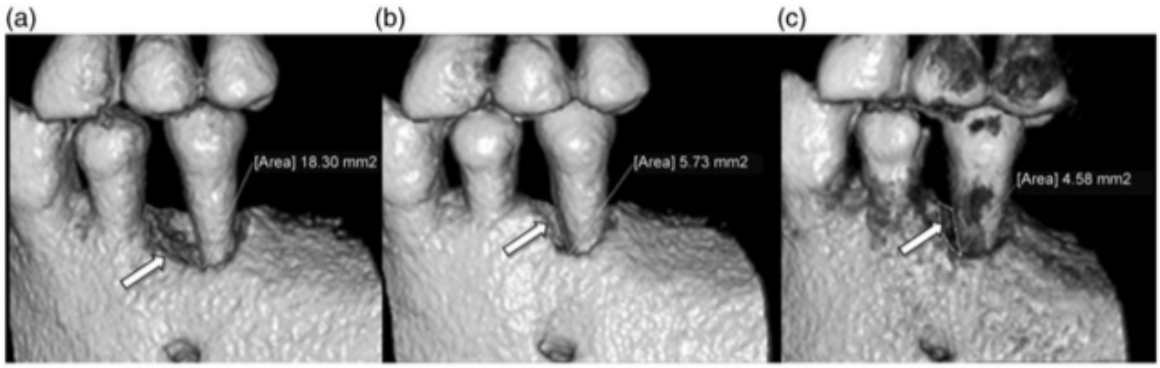

Figura 16

Cone-beam TAC del área del injerto (34-35); área inicial del defecto (18,30 mm2) (a); (b) defecto reducido a los tres meses a 5,73 mm2 (b); defecto reducido a los seis meses después a 4,58 mm2 (c)[42]

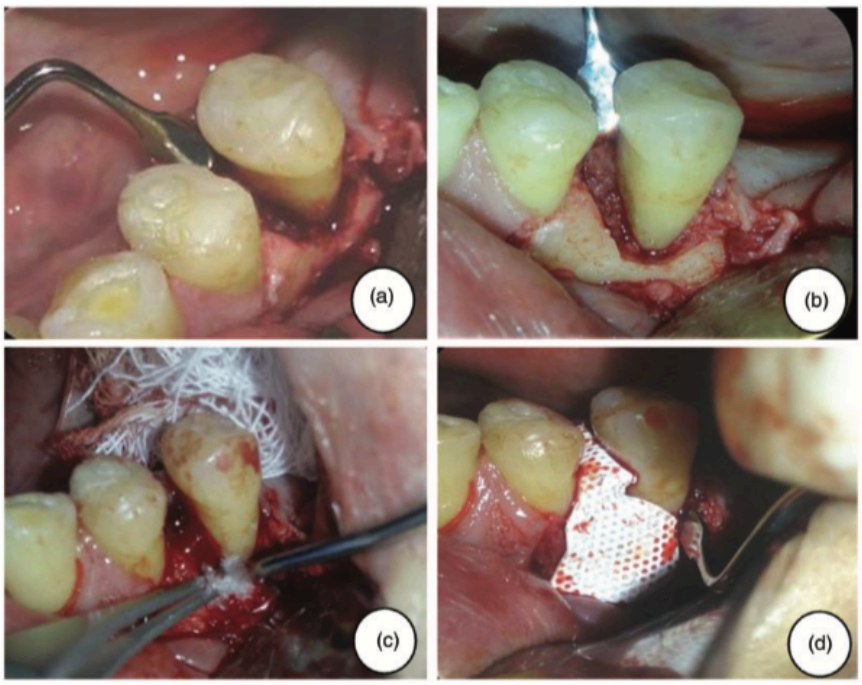

Figura 17

Defecto óseo circunferencial mesial durante la cirugía periodontal (a); elevación de colgajo y colocación de la esponja de colágeno (b); colocación de DPSCs (c); y colocación de una membrana no reabsorbible (d)[42]

En 2018 en *Journal of Clinical Periodontology* se pública un ensayo clínico de la Universidad de Turín (F.Ferrarotti y cols). El objetivo del estudio fue de evaluar las DPSCs autologas trasplantadas en defectos óseos con una esponja de colágeno en forma de concha y comprobar la eficacia clínica mejorando los parámetros clínicos y radiográficos de los defectos. Para el estudio seleccionaron 29 pacientes con periodontitis crónica diagnosticada que requerían la extracción de 1 diente vital. La muestra de pacientes fue dividida, al azar, en dos grupos; un grupo de control (15 pacientes) y un grupo de test (14 pacientes). Los dos grupos fueron sometidos a una cirugía mínimamente invasiva, para extraer el diente y para exponer el defecto óseo. Los dientes extraídos fueron procesados para poder aislar las DPSCs. La pulpa dental de los dientes se separó mecánicamente para obtener microinjertos enriquecidos en DPSCs. El grupo de test recibió una esponja de colágeno con microinjertos

(Figura 18), mientras el grupo de control recibió una esponja de colágeno sin los microinjertos (Figura 19). Durante los controles los pacientes que recibieron los microinjertos de DPSCs mostraron una significativa reducción de la profundidad de sondaje (4,9 mm vs 3,4 mm), también se pudo registrar una mejoría en la perdida de inserción (4,5 vs 2,9) y un relleno del defecto óseo (3,9 vs 1,6), con respecto al grupo de controles que recibieron la esponja de colágeno sin células. El estudio añade un valor importante a la literatura ya existente sobre el tema de las ingeniería tisular y medicina regenerativa. Se ha demostrado que la implantación de células madre autologas provenientes de la pulpa (a través de una cirugía mínimamente invasiva), en este caso, de un diente vital extraído, mejoran la potencialidad intrínseca de regeneración del defecto periodontal. Sin embargo, según los autores el ensayo ha tenido dos limitaciones; la magnitud de la muestra no fué lo suficientemente grande y los datos se han obtenido desde una única institución y en segundo lugar no se pudo establecer si ha habido regeneración periodontal, no pudiéndose hacer exámenes histológicos por razones éticas[43].

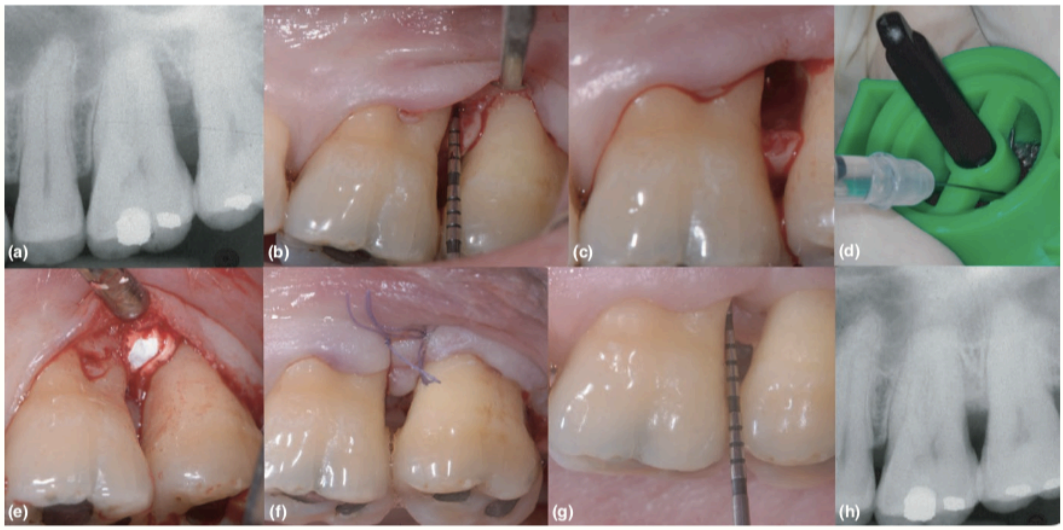

Figura 18

Secuencia clínica de cirugía mínimamente invasiva en el grupo de pacientes que ha recibido DPSCs y la esponja de colágeno. Radiografía preoperatoria del defecto óseo en distal del primer molar maxilar (a), defecto elevación del colgajo y sondaje del defecto óseo (b), visión del defecto óseo antes de la elevación de la papila interdental (c), disociación de la pulpa para la obtención de DPSCs (d), colocación del material de relleno (DPSCs y colágeno) (e), sutura (f), aspecto clínico y radiográfico 12 meses después de la cirugía (g y h)[43]

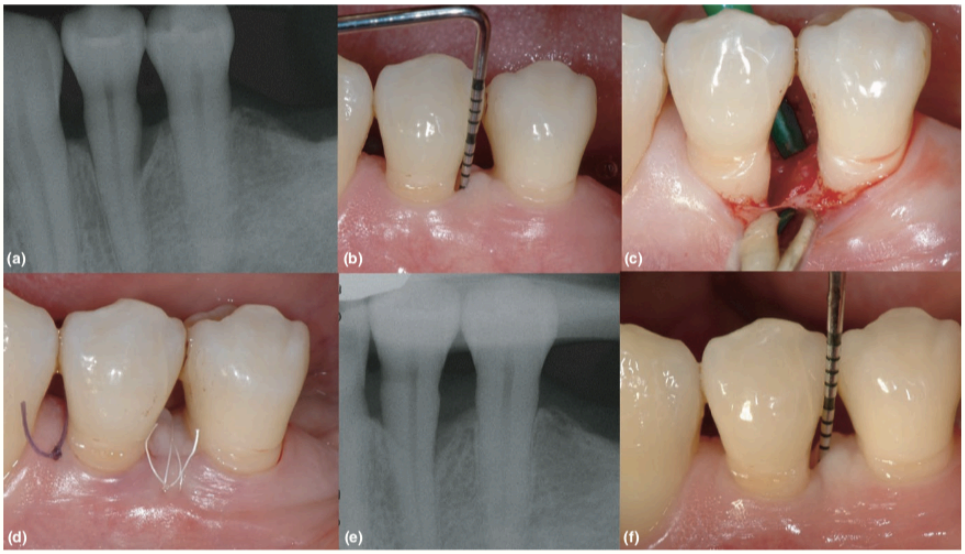

Figura 17

Secuencia clínica del grupo de control. Radiografía preoperatoria y sondaje del defecto óseo visible en la porción distal del primer premolar mandibular (a y b). Elevación del colgajo y desbridamiento del defecto (c). Sutura (d). Aspecto clínico y radiográfico 12 meses después de la intervención (e y f) [43].

Un estudio muy interesante de los autores Jeong-Ho Yun et al. sobre la combinación de PRP y BMMSCs ha sido publicado en 2014. El propósito de este estudio fue determinar la capacidad de formación ósea de las células madre mesénquimales derivadas de la médula ósea humana (BMMSC) y el plasma rico en plaquetas (PRP) cuando se aplica por separado o junto al defecto intraóseo alrededor de los implantes dentales con hidroxiapatita porosa (HA). Se generaron defectos intraóseos estandarizados de tres paredes (4 x 4 x 4 mm) en mesial de cada sitio de implante dental en cuatro perros mestizos. A continuación, los defectos se rellenaron con los siguientes materiales: HA + BMMSC (grupo HS), HA + PRP (grupo HP), HA + BMMSC + PRP (grupo HSP) y HA solo (grupo HA). El nivel de densidad ósea y osteointegración (contacto hueso-implante [BIC]) en los defectos óseos alrededor de los implantes se evaluó mediante análisis histológico e histométrico a las 6 y 12 semanas después de la colocación de los implantes. Grupos HA, HS, HP y HSP en general mostraron un aumento en la densidad ósea y BIC entre las 6 y 12 semanas, excepto BIC en el grupo HS. Aunque no se encontraron diferencias estadísticamente significativas entre los grupos HA, HS, HP y HSP ($p > 0.05$), el nivel más alto de densidad ósea y BIC se observó en el grupo HSP después del período de curación de 12 semanas. Además, el nivel de maduración ósea fue mayor en el grupo HSP que en los otros grupos según se determinó histológicamente. Los hallazgos de este estudio preliminar sugieren que las BMMSC y PRP combinados con el HA pueden proporcionar efectos terapéuticos adicionales sobre la regeneración ósea y mejorar la osteointegración en los defectos óseos alrededor de los implantes dentales. Los presentes hallazgos sugieren que las BMMSC y el PRP tienen un efecto positivo sobre la regeneración ósea para la osteointegración de implantes dentales en un modelo de defecto intraóseo de tres paredes como material de injerto óseo. Sin embargo, se requieren más estudios para

dilucidar qué elementos de BMMSC y PRP contribuyen a la formación de una calidad de hueso aún mejor de la observada en el grupo HSP (Figuras 20-21) [44].

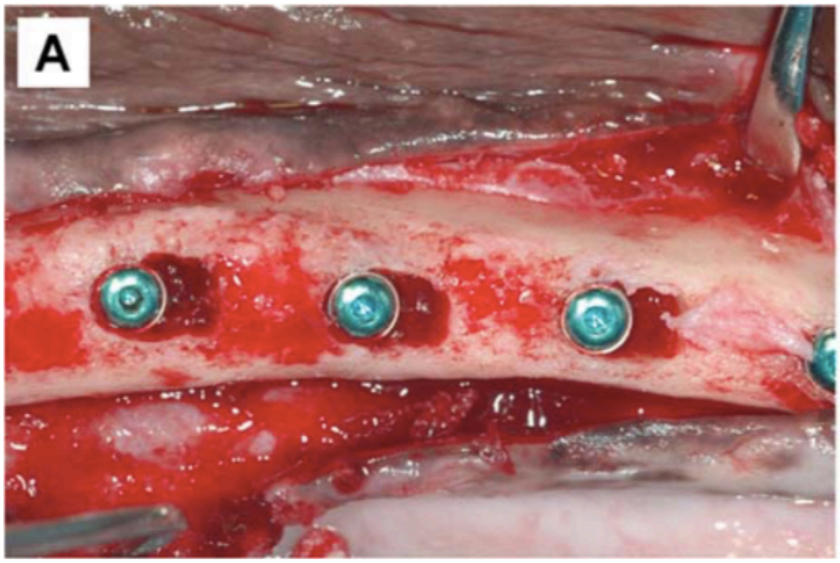

Figura 18

Fotografías clínicas del procedimiento quirúrgico. 12 semanas después de la extracción de los cuatro premolares y primeros molares mandibulares, generación de un defecto intraóseo de tres paredes (tamaño 4 x 4 x 4 mm) en el lado mesial del orificio de perforación preparado para la fijación del implante [44]

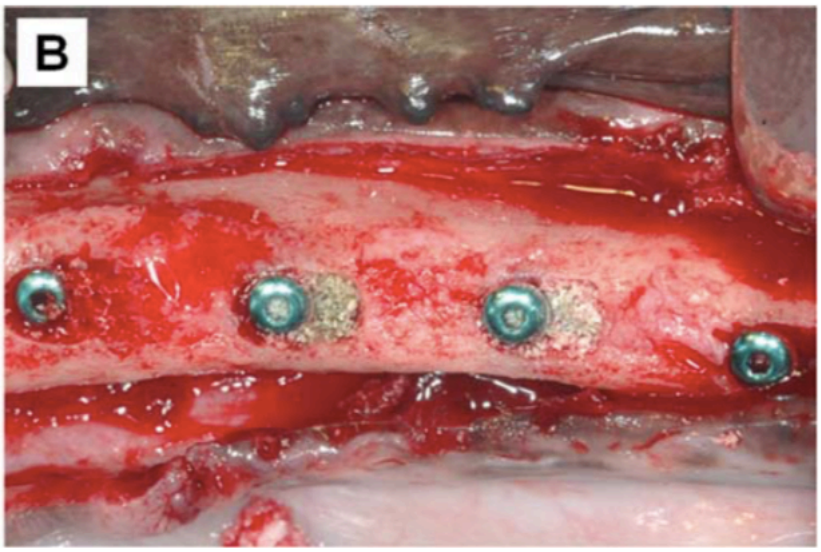

Figura 19

Fotografías clínicas del procedimiento quirúrgico. Después de colocar cuatro implantes dentales a cada lado de la mandíbula, se rellenaron materiales de injerto en el área del defecto óseo [44]

Muestra	Tratamiento	Resultados	Referencias
8	Pacientes diagnosticados con necesidad de aumento óseo antes de la colocación de implantes dentales fueron tratados con β-TCP o una esponja de atelocolágeno con BMMSCs.	BMMSCs se han utilizado de forma segura y con pocos signos inflamatorios. Han demostrado gran potencial osteogénico. Primer estudio clínico en humanos de regeneración del hueso alveolar utilizando BMMSCs	41
1	DPSCs de un diente temporal de un donante de 7 años ,se procesaron por vía enzimática y se centrifugaron y luego colocadas en una esponja de colágeno en el área del premolar inferior izquierdo de un paciente de 61 años con enfermedad periodontal	A los 3 y 6 meses: disminución de la movilidad dentaria, de profundidad de bolsas periodontales y del área del defecto óseo. Aumento de la densidad mineral ósea	42
29	La pulpa dental del diente extraído ha sido disociada mecánicamente para obtener microinjertos ricos en DPSC autólogas. Los sitios de prueba (n = 15) se llenaron con microinjertos sembrados sobre una esponja de colágeno, mientras que los sitios de control (n = 14) solo con esponja de colágeno.	La aplicación de DPSC ha mejorado significativamente los parámetros clínicos de la regeneración periodontal un año después del tratamiento.	43
4	Defectos óseos (en perros) rellenados con: HA + BMMSC (grupo HS), HA + PRP (grupo HP), HA + BMMSC + PRP (grupo HSP) y HA solo (grupo HA)	BMMSC +PRP+ HA pueden proporcionar efectos terapéuticos adicionales sobre la regeneración ósea y mejorar la osteointegración en los defectos óseos alrededor de los implantes dentales	44

Tabla 5

Aplicaciones de células madre en la enfermedad periodontal.

5.2.5 Opiniones y experiencias de los odontólogos sobre la efectividad de las células madre como tratamiento de la enfermedad periodontal ¿Qué dice la experiencia?

Con la realización de la encuesta se han obtenido 76 respuestas. El 100% de los odontólogos que han respondido al cuestionario ha leído y aceptado las condiciones contenidas en el consentimiento informado (Figura 6). La mayoría de los odontólogos que han participado a la encuesta tiene una edad comprendida entre los 30-40 años de edad, el 31,6%. El 21,5% tiene más de 50 años, el 17,1% entre 25-30 años, el 15,8% entre 40-50 años y el 14,5% entre 20-25 años (Figura 7). A la pregunta "*Conoce/se ha informado sobre estudios basados en la aplicación de células madre en el tratamiento de la enfermedad periodontal?*" (Figura 8) el 35,5% (27 respuestas) ha contestado "*No, nunca me he informado pero el tema me interesa y lo haré en un futuro*". El 32,9% (25 respuestas) ha contestado "*Si, me interesa el tema y me mantengo informado*". El 31,6% (24 respuestas) ha contestado "*Si, conozco el tema el líneas generales*". La respuesta "*No, no me interesa*" a tenido 0 contestaciones. A la pregunta "*Ha participado en estudios sobre el uso de células madre en el tratamiento de la enfermedad periodontal o ha aplicado tratamientos de este tipo en algún paciente?"* el 90,8% (68 respuestas) ha respuesto negativamente y solo un 9,3% (7 respuestas) afirmativamente (Figura 9). De los 7 que han participado en estudios sobre las células madre, el 100% ha participado en la publicación de revisiones bibliográficas (systematic, narrative, meta-analysis reviews), el 14,3% (1 respuesta) también en la publicación artículos originales, el 14,3%(1 respuesta) en la publicación de clinical cases/ case report y el 25% (2 respuestas) en la publicación de clinical trial/ randomized clínica trial (Figura 10). En la pregunta "*Que resultados se han reportado en los estudios o que opinión le merecen estos tratamientos? (según su experiencia clínica o según sus conocimientos del tema)"* se han obtenido en total

36 respuestas; el 50% (18 respuestas) ha contestado "*Los resultados demuestran que el potencial terapéutico del uso de células madre en el tratamiento de la enfermedad es muy elevado*" el 25% (9 respuestas) han contestado "*Los resultados demuestran que funcionan muy bien pero no en todos los pacientes"*, el 19,4 (7 respuestas) han contestado "*Los resultados demuestran que no hay beneficio en el uso de células madre en el tratamiento de la enfermedad periodontal*". El 2,8% (1 respuesta) "*Los resultados obtenidos no han sido suficientes para traer conclusiones sobre los efectos del utilizo de células madre en la enfermedad periodontal"* y el restante 2,8% (1 respuesta) ha contestado "*Los resultados son positivos pero no ofrecen una ventaja muy significativa respecto a otras terapias*" (Figura 11). En la última pregunta "*Según sus experiencias sobre los tratamientos clásicos de la enfermedad periodontal y según los actuales conocimientos sobre el potencial terapéutico de las células madre, considera útil la investigación sobre el uso de estas ultimas en el mundo de la Periodoncia?"* (Figura 12) el 61,8% (47 respuestas) ha contestado *"Si, me parecen útiles para nuevos avances en la ingeniería tisular y algunas terapias ya es posible aplicarlas clínicamente"* y la restante parte, el 38,2% (29 respuestas) ha contestado "*Si, son útiles pero falta desarrollarlos muchísimo mas para poder aplicarse a la población general que lo necesite"*, la respuesta "*No, pienso que los tratamientos clásicos son suficientes en el tratamiento de la enfermedad periodontal"* ha tenido 0 contestaciones. La opinión de los odontólogos que han contestado a los cuestionarios ha sido muy importante a la hora de recoger datos sobre la opinión actual de las terapias basadas en células madre. Según la mayoría, el 61,8%, ya es posible aplicar algunas terapias basadas en células madre clínicamente aunque el dato recogido por el 38,2% es al mismo tiempo, muy relevante. Esta encuesta, aunque puede dar una idea general, sin embargo, presenta unos limites dado el

numero total de odontólogos que han participado, de base no muy elevado y siendo muy bajo el porcentaje que ha realizado estudios experimentales y que puede entonces tener una idea más clara sobre posibles resultados.

5.2.6 Perspectivas futuras de las células madre en odontología regenerativa

La regeneración de un diente vital entero es el objetivo ultimo de la odontología y la línea de investigación que en el futuro permita el remplazo de un diente que se ha perdido. Existen dos diferentes enfoques para crear un bio-diente: solo con células o células y mediador biológico. El proceso de regeneración basado solo en células comienza con la obtención del germen del diente utilizando células mesénquimales y epiteliales (derivadas del embrión y de iPSCs). Estas células pueden derivan de tejidos dentales y no dentales[(21)]. Oshima y cols. han describido un protocolo para la regeneración de un diente tridimensional bio-ingenierizado, en ratas, a través el uso de células epiteliales y mesénquimales derivadas del germen dental. El diente ha sido generado de forma ectópica, conteniendo LPD y hueso alveolar. El diente ha sido trasplantado en la mandíbula mediante un injerto óseo. El diente bio-ingenierizado ha conseguido realizar las normales funciones fisiológicas normales incluyendo las funciones masticatorias y de propioceptiva. El experimento ha sido conseguido en ratas (Figura 22)[(45)].

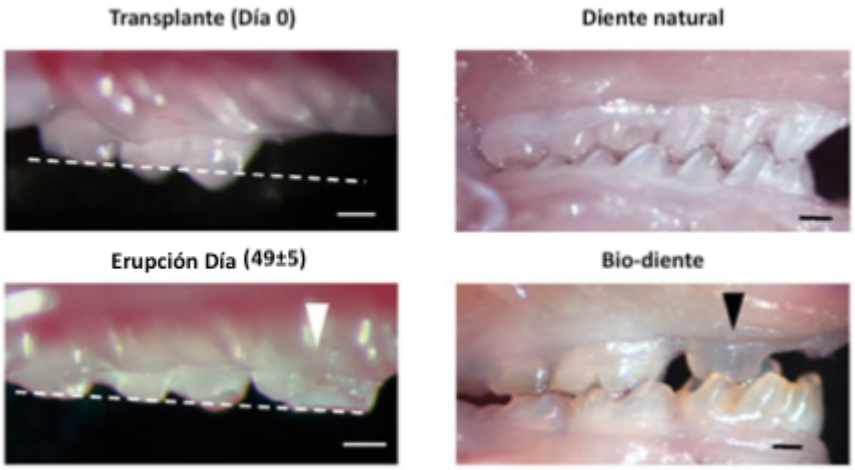

Figura 20

Evolución del bio-diente ingenierizado por Oshima y cols [45] .

Varios estudios proponen protocolos de creación de modelos de bio-raices en animales, como el de Sonoyama y cols. en mini cerdos. Para el caso, se han utilizado células madre SCAPs y PDLSCs y los resultados han traído a conclusiones positivas en cuanto aplicaciones clínicas predecibles[46]. También un estudio realizado por Wei y cols. ha traído a resultados muy similares, sin embargo con células madre diferentes (células madre dentales alógenicas)[47]. Gault et al. En 2010 han evaluado la hipótesis de sembrar un implante en titanio con PDLSCs, creando así un bio-implante y llamándolo "ligaplant". A través la implantación de este implante sembrado con células madre en un alveolo post extractivo se ha visto, en el *follow up*, la regeneración de tejido óseo nuevo y de LPD. En esta investigación se ha demostrado el potencial de la aplicación de un implante bio-osteo integrado (integrado al hueso a través del ligamento), y las ventajas sobre la osteointegración de los implantes. A pesar de esto, en la superficie del implante de titanio no hay cemento, lo que impide una fijación de las fibras colágenas que no pueden estar

como en una raíz de un diente natural. Se concluye, entonces, que el "ligaplant" no puede funcionar completamente como un verdadero diente[48]. Para llegar a resultados aceptables para que sean aplicados se necesita mucha investigación todavía y para que pueda ser regenerado un diente entero o pueda ser substituido por los bio-implantes habrá que resolver las siguientes cuestiones:

- Cual combinación celular es más conveniente para ser aplicadas en humanos?
- Conocer las posibles interacciones entre las células.
- Determinar la predictibilidad de la forma del nuevo diente.
- Definir la tumorigenicidad y la immunogenicidad de las células, ya que las capas celulares se obtienen de células embrionarias y de iPSCs (y los genes alterados en su obtención son factores de transcripción potencialmente relacionados con tumores) [21].

6. CONCLUSIONES

1. El uso de las células madre en las terapias periodontales ha llegado a resultados impresionantes. En los casos de implantaciones de células madre de natura autógena, el paciente es tanto el donador como el receptor, eliminando así el riesgo de rechazo inmunológico, una ventaja enorme. Las futuras investigaciones basadas en MSCs como enfoques terapéuticos para la regeneración de los tejidos periodontales deberá considerar los siguientes problemas:
    - La supervivencia de las células y la expresión de las capacidades de proliferación.
    - Tratamientos pre-cultivados y terapias combinadas con mediadores biológicos.
    - Controlo sobre posibles migraciones, falta de diferenciación y tumorigenicidad.
    - Interacción entre huésped y MSC trasplantadas.

- El procedimiento de obtención de algunas células (como las BMMSCs) conlleva un cierto grado de morbilidad que puede generar molestias para los pacientes.

2. En los últimos 20 años se ha investigado mucho sobre las diferentes células madre, sobre sus distintas orígenes y sobre sus diferentes capacidades de regeneración. Se ha investigado en modelos animales, en laboratorios y en humanos. Gracias a estas investigaciones se ha podido demonstrar que las células madre son un componente fundamental para la ingeniería tisular, gracias a sus propiedades regenerativas y inmunomoduladoras.

3. Los resultados procedentes de los cuestionarios refieren un dato importante sobre la opinión actual de los odontólogos que han contestado a la encuesta. Algunas terapias es posible aplicarlas clínicamente aunque merece la pena investigar más para poder encontrar protocolos más seguros para poderlos aplicar a una población mayor.

4. Los nuevos tratamientos traen a resultados evidentes y optimistas, sin embargo, falta aclarar algunos conceptos y para hacerlo falta investigar más. Las nuevas tecnologías y entusiasmantes avances en materia cientifica están abriendo el camino para que la ingeniería tisular llegue a lograr la regeneración completa de los órganos, estructuralmente y funcionalmente.

7. RESPONSABILIDAD

Este trabajo trata acerca de nuevas terapias para el tratamiento de la enfermedad periodontal. Como se ha intentado explicar, es una patología que afecta a un porcentaje muy alto de la población mundial y que por eso afecta, de forma importante, a la calidad de vida de los pacientes. Trabajando e investigando más sobre nuevas y mejores terapias para tratar esta patología también se contribuye sobre una responsabilidad social muy importante, mejorar la calidad de vida de los pacientes.

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Anexos

Universidad Europea de Madrid
Grado en Odontología
Curso Académico 2020/2021

APLICACIÓN DE CELULAS MADRE EN EL TRATAMIENTO DE LA ENFERMEDAD PERIODONTAL: SITUACIÓN ACTUAL Y PERSPECTIVAS DEL FUTURO

CONSENTIMIENTO INFORMADO

En aras a dar cumplimiento al Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo, de 27 de abril de 2016, relativo a la protección de las personas físicas en lo que respecta al tratamiento de datos personales y a la libre circulación de estos datos, y siguiendo las Recomendaciones e Instrucciones emitidas por la Agencia Española de Protección de Datos (A.E.P.D.), **SE INFORMA**:

- Los datos de carácter personal solicitados y facilitados por usted, formara parte de los datos utilizados en un trabajo de fin de grado, con finalidad formativa y de investigación.
- Solo serán solicitados aquellos datos estrictamente necesarios para el objetivo antes indicado.
- Todos los datos recogidos cuentan con el compromiso de confidencialidad, con las medidas de seguridad establecidas legalmente, y bajo ningún concepto serán cedidos o tratados por terceras personas, físicas o jurídicas, sin el previo consentimiento del profesional participante en la encuesta.
- En todo momento tiene la posibilidad de ejercer los derechos de acceso, rectificación, cancelación y oposición, indicándolo por escrito a la Universidad Europea de Madrid con domicilio en Calle Tajo, s/n, 28670. Villaviciosa de Odón, Madrid.
- Agradecer la participación en este estudio que podrá servir para ampliar los conocimiento es un área de gran importancia como las terapias odontológicas en desarrollo.

Nombre y apellidos:_____

En_____, de___de 20

APLICACIÓN DE LAS CELULAS MADRE EN EL TRATAMIENTO DE LA ENFERMEDAD PERIODONTAL

Trabajo de fin de grado

1. Edad:

- Entre 20 y 25 años
- Entre 25 y 30 años
- Entre 30 y 40 años
- Entre 40 y 50 años
- Más de 50 años

2. Conoce/se ha informado sobre estudios basados en la aplicación de células madre en el tratamiento de la enfermedad periodontal?

- Si, me interesa el tema y me mantengo informado
- Si, conozco el tema en líneas generales
- No, nunca me he informado pero el tema me interesa y lo haré en un futuro
- No, no me interesa.

3. Ha participado en estudios sobre el uso de células madre en el tratamiento de la enfermedad periodontal o ha aplicado tratamientos de este tipo en algún paciente?

- Si
- No

4. Si ha respuesto afirmativamente a la pregunta anterior, en que tipo de estudio ha participado? (es posible elegir mas de una respuesta)

- Para la publicación de artículos originales
- Para la publicación de revisiones bibliográficas (systematic, narrative, meta-analysis reviews)
- Para la publicación de clinical cases/case report
- Para la publicación de clinical trial/ randomized clinical trial

5. Que resultados se han reportado en los estudios o que opinión le merecen estos tratamientos? (según su experiencia clínica o según sus conocimientos del tema)

- Los resultados demuestran que el potencial terapéutico del uso de células madre en el tratamiento de la enfermedad periodontal es muy elevado
- Los resultados son positivos pero no ofrecen una ventaja muy significativa respecto otras terapias
- Los resultados demuestran que funcionan muy bien pero no en todos los pacientes
- Los resultados demuestran que no hay beneficio en el utilizo de células madre en el tratamiento de la enfermedad periodontal
- Los resultados obtenidos no han sido suficientes para traer conclusiones sobre los efectos de la aplicación de células madre en la enfermedad periodontal

6. Según sus experiencias sobre los tratamientos clásicos de la enfermedad periodontal y según los actuales conocimientos sobre el potencial terapéutico de las células madre, considera útil la investigación sobre el uso de estas ultimas en el mundo de la Periodoncia?

- Si, me parecen útiles para nuevos avances en la ingeniería tisular y algunas terapias ya es posible aplicarlas clínicamente
- Son útiles pero falta desarrollarlos muchísimo más para poder aplicarse a la población
- general que lo necesite
- No, pienso que los tratamientos clásicos son suficientes en el tratamiento de la enfermedad periodontal.

Universidad Europea de Madrid
Grado en Odontología
Curso Académico 2020/2021

APPLICATION OF STEM CELLS IN PERIODONTAL DESEASE: STATE OF THE ART AND FUTURE PROSPECTIVE

INFORMATIVE CONSENT

In order to comply with Regulation (EU) 2016/679 of the European Parliament and of the Council, of April 27, 2016, regarding the protection of natural persons with regard to the processing of personal data and the free circulation of these data, and following the Recommendations and Instructions issued by the Spanish Agency for Data Protection (AEPD), IT IS INFORMED:

• The personal data requested and provided by the user will be part of the data used in the dissertation work for research and information purposes.

• Only the data strictly necessary for the above objectives will be requested.

• All data collected has a commitment to confidentiality, with the security measures required by law, and in no case are they transferred or processed by third parties, physical or legal, without the prior consent of the user participating in the questionnaire.

• At any time has the opportunity to exercise their rights of access, rectification, cancellation and opposition noted in writing at the European University of Madrid with address: Calle Tajo, s / n, 28670. Villaviciosa de Odón, Madrid.

• We thank you for participating in this study, which may serve to expand knowledge in an area of great importance such as new dental therapies.

Name and surname:

_____

Place_____, of___ 20

FIRM:

_____

APPLICACION OF STEM CELLS IN PERIODONTAL DESEASE: STATE OF ART AND FUTURE PROSPECTIVE

Dissertation

1. Age
   - 20-25
   - 25-30
   - 30-40
   - 40-50
   - More than 50

2. Do you know or have you ever read studies about application of stem cells in periodontal disease?

   - Yes, I know the subject and I keep myself informed
   - Yes, I know the subject but in general terms
   - No, I don't know the subject but I'm interested and I'll study about it
   - No, I'm not interested

3. Have you ever taken part in studies on the application of stem cells in periodontal disease?
   - Yes
   - No

4. If the answer to the previous question is yes, what kind of study did you participate in? (you can choose more than one option)
   - For publishing original articles
   - For the publication of reviews (systematic, narrative, metanalisis)
   - For the publication of clinical cases / case reports
   - For the publication of clinical trials / randomized clinical trials

5. What were the results reported in the studies or what opinion do these treatments deserve? (According to your clinical experience or according to your knowledge on the subject)

   - The results demonstrate a high therapeutic potential deriving from the use of stem cells in the treatment of periodontal disease
   - The results are positive but do not offer a significant advantage over other therapies.
   - The results demonstrate its effectiveness but not in all patients
   - The results show that there are no benefits in the use of stem cells in the treatment of periodontal disease
   - The results are not sufficient to establish the effect of using stem cells in the treatment of periodontal disease

6. According to your experiences regarding the classic treatment of periodontal disease and regarding the current knowledge of the therapeutic potential of stem cells, do you consider the research on the use of stem cells useful in the field of Periodontology?

- Yes, I find it useful for new advances in the field of tissue engineering and some therapies are already applicable
- Yes, I find it very useful but there is still a lot of research to apply these treatments to the population that needs it
- No, classical treatment are sufficient in the treatment of periodontal disease

Universidad Europea de Madrid
Grado en Odontología
Curso Académico 2020/2021

APPLICAZIONE DELLE CELLULE STAMINALI NEL TRATTAMENTO DELLA MALATTIA PARODONTALE: SITUAZIONE ATTUALE E PROSPETTIVE FUTURE

CONSENSO INFORMATO

Al fine di ottemperare al Regolamento (UE) 2016/679 del Parlamento Europeo e del Consiglio, del 27 aprile 2016, relativo alla protezione delle persone fisiche con riguardo al trattamento dei dati personali e alla libera circolazione degli stessi dati, e seguendo le Raccomandazioni e le Istruzioni emanate dall'Agenzia Spagnola per la Protezione dei Dati (A.E.P.D.), **SI INFORMA:**

• I dati personali richiesti e forniti dall'utente faranno parte dei dati utilizzati nella tesi di laurea con finalità di ricerca e di informazione.

• Verranno richiesti solo i dati strettamente necessari per gli obbiettivi sopra indicati.

• Tutti i dati raccolti hanno l'impegno di riservatezza, con le misure di sicurezza previste dalla legge, e in nessun caso vengono trasferiti o trattati da terzi, fisici o legali, senza il previo consenso dell'utente che partecipa al questionario.

• In qualsiasi momento ha la possibilità di esercitare i diritti di accesso, rettifica, cancellazione e opposizione, indicandolo per iscritto all'Università Europea di Madrid con indirizzo:
Calle Tajo, s / n, 28670. Villaviciosa de Odón, Madrid.

• Si ringrazia per la partecipazione a questo studio, che potrà servire per ampliare le conoscenze in un'area di grande importanza come le nuove terapie odontoiatriche.

Nome e Cognome:_____

In_____, il___del 20

FIRMA: _____

APPLICAZIONE DELLE CELLULE STAMINALI NEL TRATTAMENTO DELLA MALATTIA PARODONTALE: SITUAZIONE ATTUALE E PROSPETTIVE FUTURE

Tesi di Laurea

1. Età

- 20-25
- 25-30
- 30-40
- 40-50
- Oltre 50

2. Conosce/ha letto studi sull'applicazione delle cellule staminali nel trattamento della malattia parodontale?

- Si, conosco l'argomento e mi mantengo aggiornato
- Si, conosco l'argomento ma in linee generali
- No, non sono informato ma l'argomento mi interessa e mi informerò in futuro
- No, non mi interessa

3. Ha preso parte in studi sull'applicazione delle cellule staminali nel trattamento della malattia parodontale o ha mai applicato questi trattamenti su pazienti?

- Si
- No

4. In caso di risposta affermativa alla precedente domanda, in che tipo di studio ha partecipato? (è possibile scegliere più di una opzione)

- Per la pubblicazione di articoli originali
- Per la pubblicazione di review (systematic, narrative, metanalisis)
- Per la pubblicazione di clinical cases/case report
- Per la pubblicazione di clinical trial/ randomized clinical trial

5. Quali sono stati i risultati riportati negli studi o che opinione meritano questi trattamenti?(secondo la sua esperienza clinica o secondo le sue conoscenze in materia)

- I risultati dimostrano un alto potenziale terapeutico derivante dall'utilizzo delle cellule staminali nel trattamento della malattia parodontale
- I risultati sono positivi ma non offrono un vantaggio significativo rispetto ad altre terapie.
- I risultati ne dimostrano l'efficacia ma non in tutti i pazienti
- I risultati dimostrano che non esistono benefici nell'utilizzo delle cellule staminali nel trattamento della malattia parodontale
- I risultati non sono sufficienti a stabilire l'effetto dell'utilizzo delle cellule staminali nel trattamento della malattia parodontale

6. Secondo le sue esperienze riguardo i trattamenti classici della malattia parodontale e riguardo le attuali conoscenze del potenziale terapeutico delle cellule staminali, considera utile la ricerca sull'utilizzo di quest'ultime nel campo della Parodontologia?

- Si, la trovo utile per i nuovi avanzi nel campo dell'ingegneria tissutale e alcune terapie sono già applicabili
- Si, la trovo molto utile ma manca ancora molta ricerca per poter applicare questi trattamenti alla popolazione che lo necessita
- No, i trattamenti classici sono sufficienti nel trattamento della malattia parodontale.

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NEW CLASSIFICATION OF PERIODONTAL AND PERI-IMPLANT DISEASES

Guest editors:
Mariano Sanz y Panos N. Papapanou

new classification of periodontal and peri-implant diseases

SEPA
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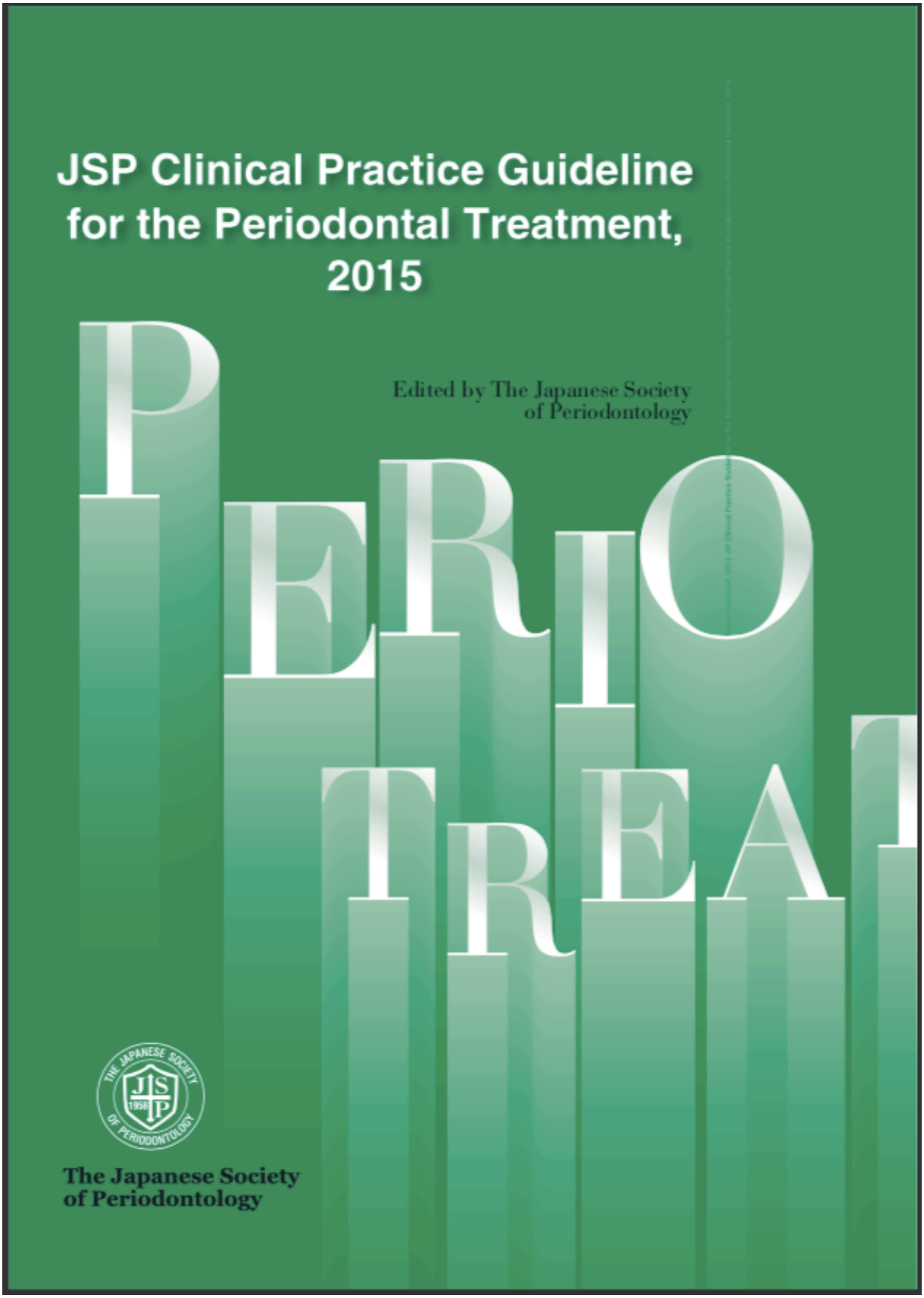
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Stem cells, tissue engineering and periodontal regeneration

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ABSTRACT

The aim of this review is to discuss the clinical utility of stem cells in periodontal regeneration by reviewing relevant literature that assesses the periodontal-regenerative potential of stem cells. We consider and describe the main stem cell populations that have been utilized with regard to periodontal regeneration, including bone marrow-derived mesenchymal stem cells and the main dental-derived mesenchymal stem cell populations: periodontal ligament stem cells, dental pulp stem cells, stem cells from human exfoliated deciduous teeth, stem cells from apical papilla and dental follicle precursor cells. Research into the use of stem cells for tissue regeneration has the potential to significantly influence periodontal treatment strategies in the future.

Keywords: Periodontium, repair, bone grafts, bioactive materials, scaffolds.

Abbreviations and acronyms: ADSC = adipose-derived stromal cell; BMP = bone morphogenetic protein; BMSC = bone marrow stromal stem cell; CFU-F = colony-forming unit fibroblast; DPSC = dental pulp stem cell; EMD = enamel matrix derivative; GFP = green fluorescent protein; IGF-I = insulin-like growth factor-I; iPS = induced pluripotent stem; ISCT = International Society for Cellular Therapy; MSC = mesenchymal stem cell; PDGF = platelet-derived growth factor; PDL = periodontal ligament; PDLSC = periodontal ligament stem cell; PRP = platelet-rich plasma; SCAP = stem cell from apical papilla; SHED = stem cell from exfoliated deciduous teeth.

INTRODUCTION

Periodontal disease is a chronic inflammatory condition of the periodontium that is characterized by irreversible destruction of the tooth attachment and its surrounding bone. The disease state, if left untreated, can lead to progressive loss of gingival tissue, periodontal ligament and supporting alveolar bone, ultimately resulting in an aesthetically and functionally compromised dentition, including premature tooth loss.[1] The pathogenesis of periodontal disease involves a complex interaction between the host's immune response to microbial colonization of the periodontal attachment, and modifying host factors, including tobacco smoking[2] and genetic susceptibility.[3] Periodontal disease has also been linked to many underlying systemic disorders such as diabetes,[4] cardiovascular disease[5] and rheumatoid arthritis.[6] Progressive periodontitis is seen in most adult human populations, with a prevalence of either moderate or severe periodontal disease in the Australian population estimated at 22.9%.[7] The consequences of untreated periodontal disease have broad ranging implications on an individual's quality of life, and thus impact upon the health system and carry a heavy economic cost. The ultimate goal of periodontal therapy relies on the achievement of complete restoration of all components of the periodontium to their original architecture and function. This entails reconstruction of gingival connective tissue, cementum, alveolar bone and periodontal ligament (PDL). In addition, formation of Sharpey's fibres, or the portion of the PDL embedded in both newly formed cementum and alveolar bone, is essential to restore appropriate connections between the tooth and its supporting tissues.[8] Current conventional techniques for the treatment of periodontal disease show a limited potential for complete periodontal regeneration. An improved understanding of periodontal biology coupled with current advances in the development of scaffolding matrices has introduced novel treatments that use cell and gene therapy to enhance periodontal tissue reconstruction and its biomechanical integration.

The periodontium

The periodontium is a complex organ consisting of two soft connective tissues (gingival and periodontal ligament) and two hard connective tissues (cementum and alveolar bone).[9]

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PERIODONTOLOGY 2000

Structure of periodontal tissues in health and disease*

ANTONIO NANCI & DIETER D. BOSSHARDT

The periodontium, defined as those tissues supporting and investing the tooth, comprises root cementum, periodontal ligament, bone lining the tooth socket (alveolar bone), and that part of the gingiva facing the tooth (dentogingival junction). The widespread occurrence of periodontal diseases and the realization that lost tissues can be repaired and, perhaps, regenerated has generated considerable interest in the factors and cells regulating their formation and maintenance. It is important to understand that each of the periodontal components has its very specialized structure and that these structural characteristics directly define function. Indeed, proper functioning of the periodontium is only achieved through structural integrity and interaction between its components.

In recent years, a number of detailed descriptions of the structural and compositional features of periodontal tissues have been published (3, 5–7, 9, 15, 17, 46, 50, 56, 58, 61); we refer the reader to these for a comprehensive description of the development, formation, and structure of periodontal tissues. The present review will focus on structure–function relationships pertinent to understanding periodontal tissue breakdown and the repair/regeneration of affected structures.

Healthy periodontal tissues

Dentogingival junction

The dentogingival junction (gingiva facing the tooth) is an adaptation of the oral mucosa that comprises epithelial and connective tissue components. The epithelium is divided into three functional compartments – *gingival*, *sulcular*, and *junctional epithelium* – and the connective tissue into *superficial* and *deep* compartments. The junctional epithelium plays a crucial role since it essentially seals off periodontal tissues from the oral environment. Its integrity is thus essential for maintaining a healthy periodontium. Periodontal disease sets in when the structure of the junctional epithelium starts to fail, an excellent example of how structure determines function.

The junctional epithelium

The junctional epithelium arises from the reduced enamel epithelium as the tooth erupts into the oral cavity. It forms a collar around the cervical portion of the tooth that follows the cementoenamel junction (Fig. 1). The free surface of this collar constitutes the floor of the gingival sulcus. Basically, the junctional epithelium is a nondifferentiated, stratified squamous epithelium with a very high rate of cell turnover. It is thickest near the bottom of the gingival sulcus and tapers to a thickness of a few cells as it descends apically along the tooth surface. This epithelium is made up of flattened cells oriented parallel to the tooth that derive from a layer of cuboidal basal cells situated away from the tooth surface that rest on a basement membrane. Suprabasal cells have a similar ultrastructure and, quite remarkably, maintain the ability to undergo cell division. The cell layer facing the tooth provides the actual attachment of the gingiva to the tooth surface by means of a structural complex called the *epithelial attachment*. This complex consists of a basal lamina-like structure that is adherent to the tooth surface and to which the superficial cell layer is attached by hemidesmosomes. The basal lamina-like structure is a specialized extracellular matrix in which typical basement membrane constituents have not been immunodetected in any significant quantity but which is

*Parts of this article are adapted from Reference 50.

Review

Therapeutic potential of dental stem cells

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Abstract

Stem cell biology has become an important field in regenerative medicine and tissue engineering therapy since the discovery and characterization of mesenchymal stem cells. Stem cell populations have also been isolated from human dental tissues, including dental pulp stem cells, stem cells from human exfoliated deciduous teeth, stem cells from apical papilla, dental follicle progenitor cells, and periodontal ligament stem cells. Dental stem cells are relatively easily obtainable and exhibit high plasticity and multipotential capabilities. The dental stem cells represent a gold standard for neural-crest-derived bone reconstruction in humans and can be used for the repair of body defects in low-risk autologous therapeutic strategies. The bioengineering technologies developed for tooth regeneration will make substantial contributions to understand the developmental process and will encourage future organ replacement by regenerative therapies in a wide variety of organs such as the liver, kidney, and heart. The concept of developing tooth banking and preservation of dental stem cells is promising. Further research in the area has the potential to herald a new dawn in effective treatment of notoriously difficult diseases which could prove highly beneficial to mankind in the long run.

Keywords

Dental stem cell, stem cell therapy, differentiation, regeneration, tissue engineering, tooth banking

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Introduction

The tooth is composed of distinct tissues including the outer mineralized enamel layer; the adjacent mineralized dentin layer; the dental pulp containing blood vessels, nerves, and mesenchymal tissue; and root structures composed of dentin, cementum, and periodontal ligament (PDL), which secure teeth to the underlying alveolar bone. Dentin contains characteristic and distinctive tubules, produced by neural crest derived dental mesenchymal stem cells called odontoblasts, which persist in mature teeth and exhibit limited regenerative capacities to form reparative dentin in response to injury or disease. The dental pulp is composed of dental mesenchymal cells, nerves, and blood vessels that thread through the root canal. Teeth develop through continuous and reciprocal interactions between cranial neural crest-derived mesenchymal stem cells (MSCs) and oral-derived epithelial stem cells during early embryogenesis.[1,2]

Stem cells can be isolated from several oral tissues such as craniofacial bone, dental pulp, PDL, dental follicle, tooth germ, apical papilla, oral mucosa, gingival, and periosteum.[3] The dental stem cells (DSCs) are post-natal stem cell populations that have MSC-like qualities, including the capacity for self-renewal and multilineage differentiation potential. These cells are derived from the neural crest, and thus have a different origin from bone-marrow-derived mesenchymal stem cells (BMMSCs), which are derived from mesoderm.[4] Among oral tissue-derived stem cells, human dental pulp stem cells (hDPSCs) have been

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Review

Mesenchymal Stem Cells of Dental Origin for Inducing Tissue Regeneration in Periodontitis: A Mini-Review

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Abstract: Periodontitis is a chronic disease that begins with a period of inflammation of the supporting tissues of the teeth table and then progresses, destroying the tissues until loss of the teeth occurs. The restoration of the damaged dental support apparatus is an extremely complex process due to the regeneration of the cementum, the periodontal ligament, and the alveolar bone. Conventional treatment relies on synthetic materials that fill defects and replace lost dental tissue, but these approaches are not substitutes for a real regeneration of tissue. To address this, there are several approaches to tissue engineering for regenerative dentistry, among them, the use of stem cells. Mesenchymal stem cells (MSC) can be obtained from various sources of adult tissues, such as bone marrow, adipose tissue, skin, and tissues of the orofacial area. MSC of dental origin, such as those found in the bone marrow, have immunosuppressive and immunotolerant properties, multipotency, high proliferation rates, and the capacity for tissue repair. However, they are poorly used as sources of tissue for therapeutic purposes. Their accessibility makes them an attractive source of mesenchymal stem cells, so this review describes the field of dental stem cell research and proposes a potential mechanism involved in periodontal tissue regeneration induced by dental MSC.

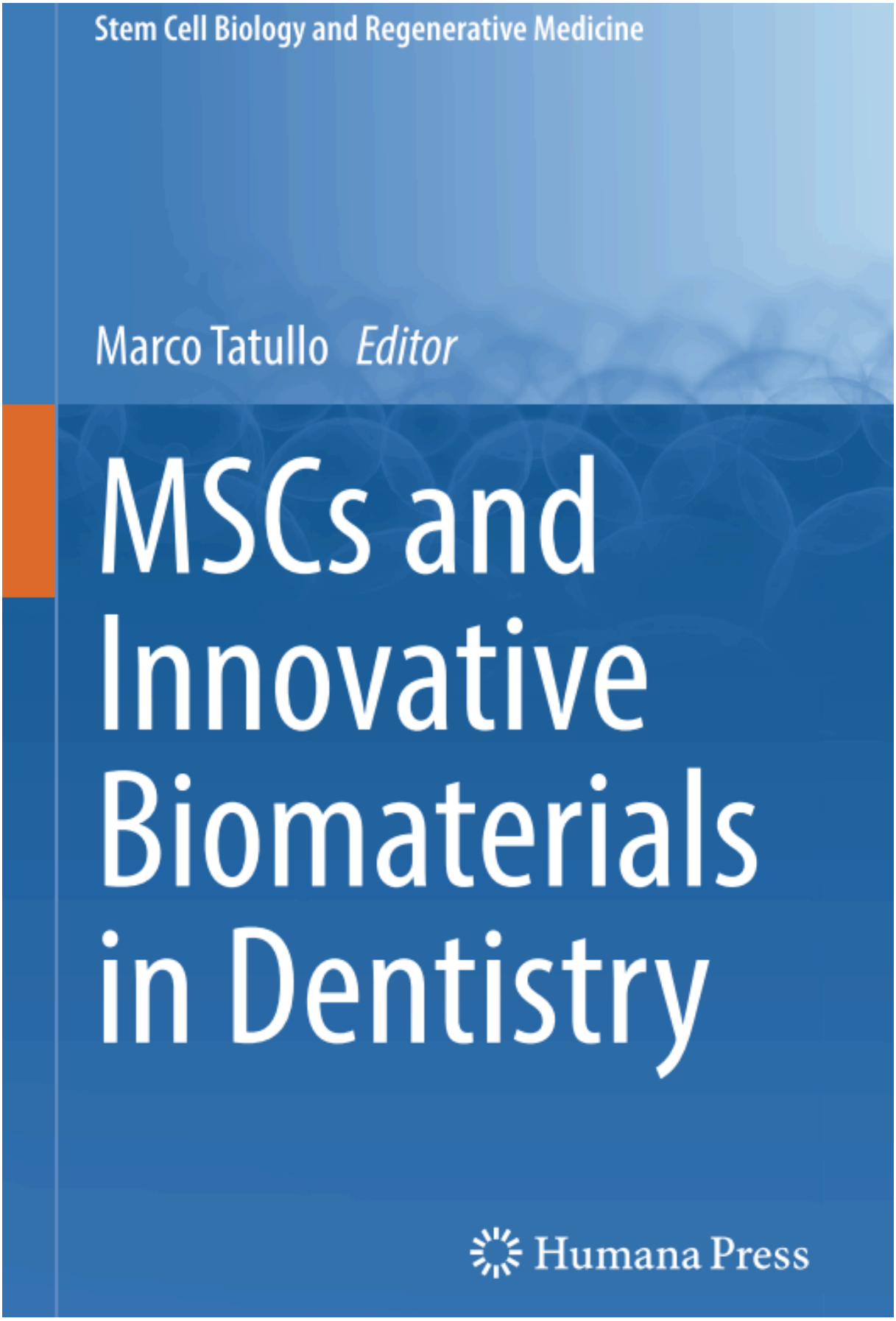
Keywords: DPSC; biological mechanism; periodontal treatment

1. Introduction

Periodontitis is a chronic inflammatory disease of the supportive tissues of the teeth. This disease is caused by specific microorganisms or groups of specific microorganisms, which result in a pathological disinsertion of the collagen fibres of the cementum; progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession, or both; and apical migration of the union epithelium [1].

When these conditions last over time, they cause the tissue to continue to be destroyed until the tooth is lost due to lack of support. This not only has repercussions at the local level that affect the chewing, phonation, and aesthetics of the patient but it is also related to other pathologies that affect quality of life [2].

Although the disease can be treated successfully in its early stages, unfortunately, it is diagnosed when it affects the periodontal ligament, which causes most patients to seek dental care when the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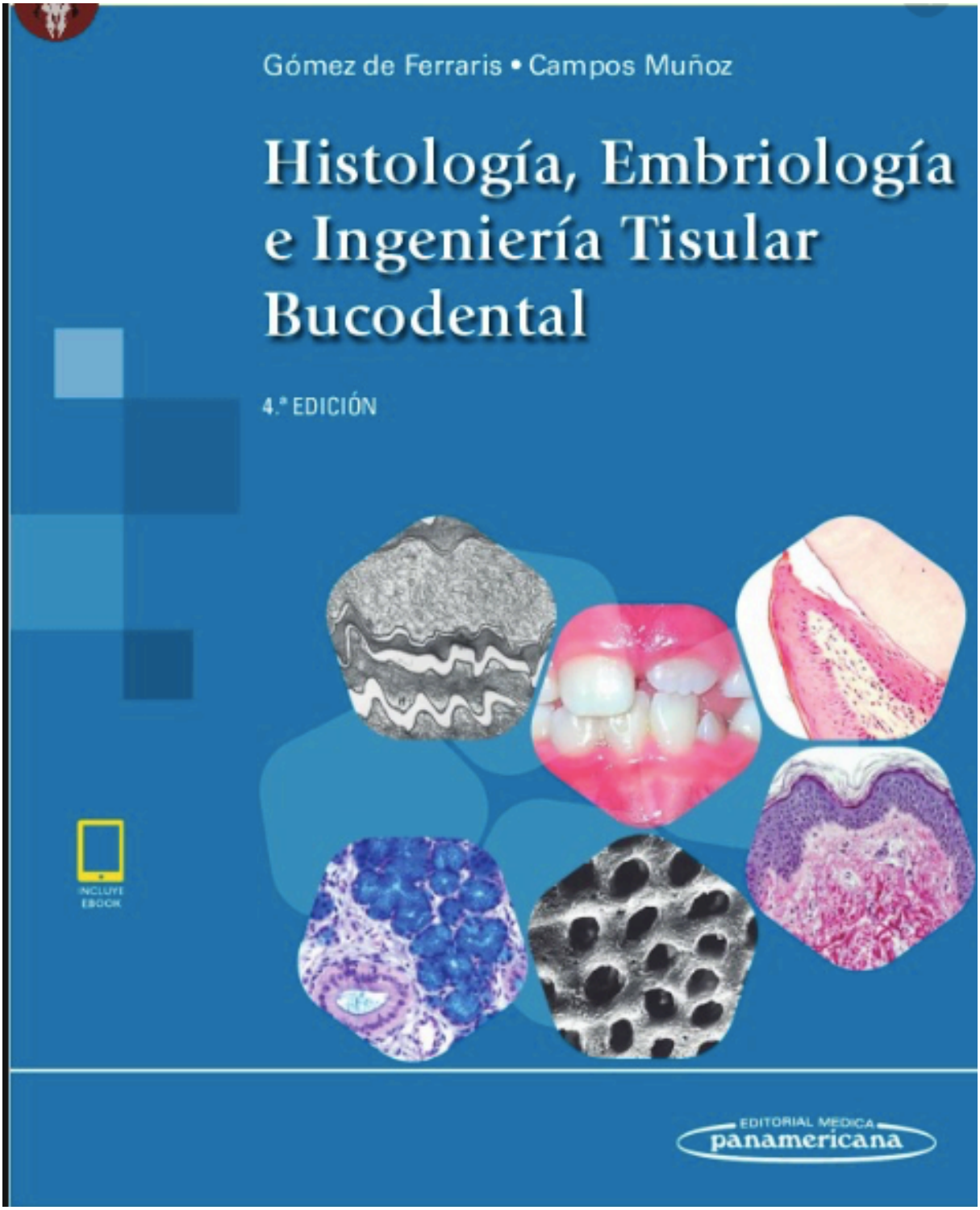
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Section 11

Consensus Report
Periodontal Diseases: Pathogenesis and Microbial Factors

The section members read the two review papers on pathogenesis and microbial factors. The section members found it necessary to include older references in the pathogenesis section. The section members, by consensus, consider the following statements to be supported by the evidence included in the two reviews and the references listed below.

PATHOGENESIS

1. What are the important destructive mechanisms in periodontal diseases?

Bacterial substances interact mainly with mononuclear phagocytic cells and fibroblasts resulting in activation and production of catabolic mediators including primarily IL-1β, PGE_2, TNFα, and IL-6. These cytokines mediate the secretion of matrix metalloproteinases (MMP). The most documented bacterial substance initiating these reactions is lipopolysaccharide (LPS) (see reference 1).

2. What are the important protective mechanisms in periodontal diseases?

Protective mechanisms are thought to fall into three categories (see reference 2):

1. Mechanical/physical mechanisms such as the epithelial barrier and the flushing action of gingival crevicular fluid flow;
2. Nonspecific factors such as PMNs and complement and specific immune factors, especially specific antibody; and
3. Rapid tissue turnover.

3. What are the important genetic factors and what is the state-of-the-art relative to assessing these genetic factors in periodontal diseases?

For the early-onset forms of periodontitis, there is good evidence from segregation analysis for involvement of a major gene effect. There is some evidence associating HLA specificities with early-onset periodontitis. The relative risk of disease associated with the presence of these specificities, however, is low compared to diseases such as ankylosing spondylitis and celiac disease. There is no evidence to date linking early onset periodontitis in diverse populations to any gene or marker loci.

In adult onset periodontitis, twin studies suggest that a significant portion of the population variance for measures of disease is attributable to genetic factors. To date there is no evidence for a major gene effect on adult onset periodontitis, nor has the disease been linked to any gene loci.

There are significant racial differences in both the prevalence of early-onset forms of periodontitis and associated host factors. It is currently unclear whether these differences are due to genetic or environmental factors.

Genetic studies in periodontology are complicated by the difficulty of defining specific

THE ROLE OF ACQUIRED IMMUNITY AND PERIODONTAL DISEASE PROGRESSION

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ABSTRACT: Our understanding of the pathogenesis in human periodontal diseases is limited by the lack of specific and sensitive tools or models to study the complex microbial challenges and their interactions with the host's immune system. Recent advances in cellular and molecular biology research have demonstrated the importance of the acquired immune system not only in fighting the virulent periodontal pathogens but also in protecting the host from developing further devastating conditions in periodontal infections. The use of genetic knockout and immunodeficient mouse strains has shown that the acquired immune response—in particular, $CD4^+$ T-cells—plays a pivotal role in controlling the ongoing infection, the immune/inflammatory responses, and the subsequent host's tissue destruction. In particular, studies of the pathogen-specific $CD4^+$ T-cell-mediated immunity have clarified the roles of: (i) the relative diverse immune repertoire involved in periodontal pathogenesis, (ii) the contribution of pathogen-associated Th1-Th2 cytokine expressions in periodontal disease progression, and (iii) micro-organism-triggered periodontal $CD4^+$ T-cell-mediated osteoclastogenic factor, 'RANK-L', which is linked to the induction of alveolar bone destruction *in situ*. The present review will focus on some recent advances in the acquired immune responses involving B-cells, $CD8^+$ T-cells, and $CD4^+$ T-cells in the context of periodontal disease progression. New approaches will further facilitate our understanding of their underlying molecular mechanisms that may lead to the development of new treatment modalities for periodontal diseases and their associated complications.

Abbreviations used in the paper are as follows: Antibody, Ab; antigen, Ag; antigen-presenting cells, APC; *Actinobacillus actinomycetemcomitans*, *A. actinomycetemcomitans* or *Aa*; β_2-microglobulin, β_2m; cytotoxic $CD8^+$ $\alpha\beta$ T-lymphocytes, CTL; dendritic cells, DC; delayed-type hypersensitivity, DTH; immunoglobulin, Ig; Fc receptor, Fc-R; interferon-γ, IFN-γ; receptor activator of NF-κB ligand, RANK-L; molecular weight, MW; *Porphyromonas gingivalis*, *P. gingivalis* or *Pg*; localized juvenile periodontitis, LJP; lipopolysaccharide, LPS; mouse mammalian tumor virus, MMTV; non-obese diabetic and severe combined immunodeficiency mice, NOD/SCID mice; osteoclast, OC; T-helper cells, Th; superantigen, SAg; transforming growth factor-β, TGF-β; secretory-IgA, s-IgA; T-cell receptor, TCR; T cytotoxic-1 cells, Tc1; and T cytotoxic-2 cells, Tc2.

Key words. Human periodontitis, acquired immunity, $CD4^+$ T-cell immune repertoire, type-1 *vs.* type-2 cytokines, osteoclastogenesis, RANK-L.

(1) Introduction

The periodontium that anchors the teeth to the jaws consists of the gingiva, periodontal ligament, cementum, and alveolar bone. It is normally in a balanced state with the periodontal microbiota in the dental plaque (biofilm). Human periodontal diseases (*i.e.*, gingivitis, periodontitis) result from heterogeneous etiologies including complex biofilm in the subgingival microenvironment, social and behavior modulations, and genetic or epigenetic traits of the host, each of which is influenced and/or modulated by the host's immune and inflammatory responses. As a result of the maturation and changes in the biofilm, mainly an increase in facultative anaerobic, Gram-negative micro-organisms (Socransky *et al.*, 1998), early vascular changes occur in the periodontium, with exudation and migration of phagocytic cells, including neutrophils and monocytes/macrophages, into the junctional epithelium and gingival sulcus, resulting in initial gingival inflammation. These changes are accompanied by increases in the size of the connective tissue infiltrated by leukocytes, loss of perivascular collagen fibers, and proliferation of junctional epithelium. During the early stage, the inflammatory infiltrate is mostly T-cells, whereas in the established lesions, B-cells become the most common inflammatory cells (Page and Schroeder, 1976). These changes signify a local alteration of immunoregulatory events in the host. The resulting cellular and fluid exudates cause further breakdown of the adjacent connective tissue and epithelium, followed by proliferation, apical migration, and lateral extension of the junctional epithelium. All of these alterations contribute to periodontal pocket formation. The pathogenic species present in the subgingival biofilm release an array of virulence factors that can evade anti-bacterial host defense mechanisms and then cause damage to the host tissue *via* immune/inflammatory interactions, which typically consist of neutrophils, monocytes/macrophages, dendritic cells (DCs), T-cells, and predominantly IgG-producing plasma cells. As the disease proceeds to more advanced stages, tissue destruction involves significant alveolar bone resorption and continuing loss of the collagen needed for tissue attachment. Widespread manifestations of inflammatory and pathological responses associated with periods of quiescence and active exacerbation become evident (Nabers *et al.*, 1988; Papapanou *et al.*, 1989; Beck, 1996). Further study and understanding of the underlying pathological mechanisms await more sensitive measurement or methods that differentiate between active and quiescent disease stages.

Despite the production of many virulence factors—such as proteases, metabolic and toxic by-products, enzymes, and lipopolysaccharides (LPS) from subgingival micro-organisms—

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2017 WORLD WORKSHOP

Staging and grading of periodontitis: Framework and proposal of a new classification and case definition

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The proceedings of the workshop were jointly and simultaneously published in the *Journal of Periodontology* and *Journal of Clinical Periodontology*.

Abstract

Background: Authors were assigned the task to develop case definitions for periodontitis in the context of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. The aim of this manuscript is to review evidence and rationale for a revision of the current classification, to provide a framework for case definition that fully implicates state-of-the-art knowledge and can be adapted as new evidence emerges, and to suggest a case definition system that can be implemented in clinical practice, research and epidemiologic surveillance.

Methods: Evidence gathered in four commissioned reviews was analyzed and interpreted with special emphasis to changes with regards to the understanding available prior to the 1999 classification. Authors analyzed case definition systems employed for a variety of chronic diseases and identified key criteria for a classification/case definition of periodontitis.

Results: The manuscript discusses the merits of a periodontitis case definition system based on Staging and Grading and proposes a case definition framework. Stage I to IV of periodontitis is defined based on severity (primarily periodontal breakdown with reference to root length and periodontitis-associated tooth loss), complexity of management (pocket depth, infrabony defects, furcation involvement, tooth hypermobility, masticatory dysfunction) and additionally described as extent (localized or generalized). Grade of periodontitis is estimated with direct or indirect evidence of progression rate in three categories: slow, moderate and rapid progression (Grade A-C). Risk factor analysis is used as grade modifier.

Conclusions: The paper describes a simple matrix based on stage and grade to appropriately define periodontitis in an individual patient. The proposed case definition extends beyond description based on severity to include characterization of biological features of the disease and represents a first step towards adoption of precision medicine concepts to the management of periodontitis. It also provides the necessary framework for introduction of biomarkers in diagnosis and prognosis.

KEYWORDS

aggressive periodontitis, biomarkers, case definition, chronic periodontitis, classification, clinical attachment loss, diagnosis, furcation involvement, grade A periodontitis, grade B periodontitis, grade C

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DIAGNÓSTICO Y TRATAMIENTO PERIODONTAL

Directores invitados:
José Javier Echeverría y Niklaus P. Lang

diagnóstico y tratamiento periodontal

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REVIEW ARTICLE

Application of platelet-rich plasma with stem cells in bone and periodontal tissue engineering

Gabriela Fernandes[1] and Shuying Yang[1,2,3]

Presently, there is a high paucity of bone grafts in the United States and worldwide. Regenerating bone is of prime concern due to the current demand of bone grafts and the increasing number of diseases causing bone loss. Autogenous bone is the present gold standard of bone regeneration. However, disadvantages like donor site morbidity and its decreased availability limit its use. Even allografts and synthetic grafting materials have their own limitations. As certain specific stem cells can be directed to differentiate into an osteoblastic lineage in the presence of growth factors (GFs), it makes stem cells the ideal agents for bone regeneration. Furthermore, platelet-rich plasma (PRP), which can be easily isolated from whole blood, is often used for bone regeneration, wound healing and bone defect repair. When stem cells are combined with PRP in the presence of GFs, they are able to promote osteogenesis. This review provides in-depth knowledge regarding the use of stem cells and PRP *in vitro*, *in vivo* and their application in clinical studies in the future.

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INTRODUCTION

Regenerating the lost bone is of primary concern in diseases and conditions involving bone loss, such as periodontitis, tumors, fractures, and bony defects.[1] Autogenous bone has long been held as the gold standard of bone grafting materials; however, donor site morbidity, difficulty in obtaining it, and the prolonged healing time are its limitations.[2] In recent years, autologous bone has been administered for the regeneration of bony defects and structures.[3] But, the risk of disease transmission and foreign body immune reaction associated with it is high.[4] In addition, synthetic bone grafting materials have been created and produced to mimic bony structure and cellular morphology along with promoting osteoconduction;[5] however, the primary expenses involved in fabricating and manufacturing these graft materials preclude their extensive application.[6] Hence, it is imperative to advocate and implement newer techniques and entities in order to overcome these limitations.[7] Bone tissue engineering is the field of medicine that involves the regeneration and replacement of the lost bony tissue and structure.[4] Due to the increasing demand and the paucity of the presently existing bone grafts, it has now become imperative to devise novel materials that can achieve excellent regeneration as well as reduce the drawbacks of the presently existing grafting materials.[8] It is very important to harness the potential of cellular and molecular technology in order to develop newer grafting materials and exploit its practical applications.[9–11]

A high volume of research in bone tissue engineering has been devoted to adult stem cells, which can be isolated from tissues such as a bone marrow or adipose tissue. Mesenchymal stem cells (MSCs) have been identified as the cells which adhere to plastic, lack of expression and absence of the hematopoietic and endothelial markers and their ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages.[12–14] Adult bone marrow-derived MSCs (BMSCs) have been the focus of most studies due to the inherent potential of these cells to differentiate into various cell types. In the past decade, MSCs have been employed in the regeneration of bone, especially because of its potential to differentiate into an osteogenic

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Stem Cell Research & Therapy

RESEARCH Open Access

Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice

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Abstract

Background: Periodontitis, one of the most prevalent infectious diseases in humans, results in the destruction of tooth-supporting tissues. The purpose of the present study is to evaluate the effect of cell injection and cell sheet transplantation on periodontal regeneration in a swine model.

Methods: In the present study, human dental pulp stem cells (hDPSCs) were transplanted into a swine model for periodontal regeneration. Twelve miniature pigs were used to generate periodontitis with bone defects of 5 mm in width, 7 mm in length, and 3 mm in depth. hDPSCs were obtained for bone regeneration using cell injection or cell sheet transplantation. After 12 weeks, clinical, radiological, and histological assessments of regenerated periodontal tissues were performed to compare periodontal regeneration treated with xenogeneic cell injection and cell sheet implantation.

Results: Our study showed that translating hDPSCs into this large animal model could significantly improve periodontal bone regeneration and soft tissue healing. After 12 weeks, both the hDPSC sheet treatment and hDPSC injection significantly improved periodontal tissue healing clinically in comparison with the control group. The volume of regenerative bone in the hDPSC sheet group (52.7 ± 4.1 mm^3) was significantly larger than in the hDPSC injection group (32.4 ± 5.1 mm^3) ($P < 0.05$). The percentage of bone in the periodontium in the hDPSC injection group was 12.8 ± 4.4 %, while it was 17.4 ± 5.3 % in the hDPSC sheet group ($P < 0.05$).

Conclusion: Both hDPSC injection and cell sheet transplantation significantly regenerated periodontal bone in swine. The hDPSC sheet had more bone regeneration capacity compared with hDPSC injection.

Keywords: Dental pulp stem cells, Cell injection, Cell sheet, Periodontal bone regeneration

Background

Periodontitis, one of the most prevalent infectious diseases in humans, results in the destruction of tooth-supporting tissues such as bone, periodontal ligaments, and cementum [1]. Several regenerative approaches, including guided tissue regeneration [2], application of biological mediators such as enamel matrix derivative (EMD) [3], and other scaffold-based techniques [4], were proposed to treat periodontal disease, and favorable results were obtained in clinical trials and animal models. Based on recent progress in tissue engineering, ex vivo expanded mesenchymal stem cells (MSCs) are used in regenerative medicine because of their potential to differentiate into multiple lineages [5–9]. Previously, we generated a swine model of periodontitis [10]. In this model, we induced significant periodontal tissue regeneration using periodontal ligament stem cells (PDLSCs)

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Review Article

Comparison of full-mouth disinfection and quadrant-wise scaling in the treatment of adult chronic periodontitis: a systematic review and meta-analysis

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Scaling and root planing are widely considered as effective methods for treating chronic periodontitis. A meta-analysis published in 2008 showed no statistically significant differences between full-mouth disinfection (FMD) or full-mouth scaling and root planing (FMS) and quadrant scaling and root planing (Q-SRP). The FMD approach only resulted in modest additional improvements in several indices. Whether differences exist between these two approaches requires further validation. Accordingly, a study was conducted to further validate whether FMD with antiseptics or FMS without the use of antiseptics within 24 h provides greater clinical improvement than Q-SRP in patients with chronic periodontitis. Medline (via OVID), EMBASE (via OVID), PubMed and CENTRAL databases were searched up to 27 January 2015. Randomized controlled trials comparing FMD or FMS with Q-SRP after at least 3 mo were included. Meta-analysis was performed to obtain the weighted mean difference (WMD), together with the corresponding 95% confidence intervals. Thirteen articles were included in the meta-analysis. The WMD of probing pocket depth reduction was 0.25 mm ($p < 0.05$) for FMD vs. Q-SRP in single-rooted teeth with moderate pockets, and clinical attachment level gain in single- and multirooted teeth with moderate pockets was 0.33 mm ($p < 0.05$) for FMD vs. Q-SRP. Except for those, no statistically significant differences were found in the other subanalyses of FMD vs. Q-SRP, FMS vs. Q-SRP and FMD vs. FMS. Therefore, the meta-analysis results showed that FMD was better than Q-SRP for achieving probing pocket depth reduction and clinical attachment level gain in moderate pockets. Additionally, regardless of the treatment, no serious complications were observed. FMD, FMS and Q-SRP are all effective for the treatment of adult chronic periodontitis, and they do not lead to any obvious discomfort among patients. Moreover, FMD had modest additional clinical benefits over Q-SRP,

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Key words: chronic periodontitis; full-mouth debridement; quadrant-wise scaling; root planing

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Regenerative Medicine for Periodontal and Peri-implant Diseases

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Abstract

The balance between bone resorption and bone formation is vital for maintenance and regeneration of alveolar bone and supporting structures around teeth and dental implants. Tissue regeneration in the oral cavity is regulated by multiple cell types, signaling mechanisms, and matrix interactions. A goal for periodontal tissue engineering/regenerative medicine is to restore oral soft and hard tissues through cell, scaffold, and/or signaling approaches to functional and aesthetic oral tissues. Bony defects in the oral cavity can vary significantly, ranging from smaller intrabony lesions resulting from periodontal or peri-implant diseases to large osseous defects that extend through the jaws as a result of trauma, tumor resection, or congenital defects. The disparity in size and location of these alveolar defects is compounded further by patient-specific and environmental factors that contribute to the challenges in periodontal regeneration, peri-implant tissue regeneration, and alveolar ridge reconstruction. Efforts have been made over the last few decades to produce reliable and predictable methods to stimulate bone regeneration in alveolar bone defects. Tissue engineering/regenerative medicine provide new avenues to enhance tissue regeneration by introducing bioactive models or constructing patient-specific substitutes. This review presents an overview of therapies (e.g., protein, gene, and cell based) and biomaterials (e.g., resorbable, nonresorbable, and 3-dimensionally printed) used for alveolar bone engineering around teeth and implants and for implant site development, with emphasis on most recent findings and future directions.

Keywords: tissue engineering, alveolar bone, gene therapy, 3D printing, growth factors, regeneration

Pathogenesis of Defects Associated with Periodontal and Peri-implant Diseases

Alveolar bone lining the socket containing teeth is remodeled continuously. This fine-tuned balance between bone resorption and bone formation is maintained by multiple cell types and signaling mechanisms (Nanci and Bosshardt 2006; Fig. 1). In susceptible individuals, the inflammatory response to bacteria can initiate the destructive process of periodontitis, leading to loss of connective tissue and bone, as well as apical migration of the junctional epithelium (Seymour et al. 2015). While disruption of subgingival microbial biofilm and resolution of periodontal inflammation can be achieved by nonsurgical therapy, restitution ad integrum cannot normally be expected, leaving a reduced periodontium and potentially residual alveolar bone defects. In more advanced cases of periodontitis, the tooth loses support and is exfoliated or extracted. To ensure masticatory function and aesthetics, replacement of missing teeth is often considered necessary. However, due to extensive previous bone resorption, alveolar bone defects may prevent correct positioning of dental implants, requiring augmentation. Following successful osseointegration, dental implants can also undergo a process of microbially driven chronic inflammation leading to bone resorption (peri-implantitis) and potentially implant loss (Carcuac and Berglundh 2014). Therefore, the clinical need for alveolar bone regeneration arises to improve long-term prognosis of teeth with periodontitis and implants affected by peri-implantitis and for the development of alveolar bone sites for implant placement. In addition to bone loss due to chronic inflammation, bone regeneration may be needed to correct defects of other origins, including trauma, tumor resection, or congenital and developmental conditions (Giannobile 2014). Efforts have been made over recent decades to predictably stimulate bone regeneration for alveolar bone defects around teeth as well as more recently in edentulous areas and around implants affected by peri-implantitis. This review highlights therapies and biomaterials used for alveolar bone engineering, with emphasis on the most recent findings and future avenues.

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Wound Healing Complications Following Guided Bone Regeneration for Ridge Augmentation: A Systematic Review and Meta-Analysis

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Hsun-Liang Chan, DDS, MS[4]/Hom-Lay Wang, DDS, MSD, PhD[5]

Purpose: *The rate of developing soft tissue complications that accompany guided bone regeneration (GBR) procedures varies widely, from 0% to 45%. The present review was conducted to investigate the rate for resorbable versus nonresorbable membranes and the timing of soft tissue complications.* ***Materials and Methods:*** *Electronic and manual literature searches were conducted by two independent reviewers using several databases, including MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, and Cochrane Oral Health Group Trials Register, for articles published through July 2015, with no language restriction. Articles were included if they were clinical trials aimed at demonstrating the incidence of soft tissue complications following GBR procedures.* ***Results:*** *Overall, 21 and 15 articles were included in the qualitative and quantitative synthesis, respectively. The weighted complication rate of the overall soft tissue complications, including membrane exposure, soft tissue dehiscence, and acute infection/abscess, into the calculation was 16.8% (95% CI = 10.6% to 25.4%). When considering the complication rate based on membrane type used, resorbable membrane was associated with a weighted complication rate of 18.3% (95% CI: 10.4% to 30.4%) and nonresorbable membrane with a rate of 17.6% (95% CI: 10.0% to 29.3%). Moreover, soft tissue lesions were reported as early as 1 week and as late as 6 months based on the included studies.* ***Conclusion:*** *Soft tissue complications after GBR are common (16.8%). Membrane type did not appear to significantly affect the complication rate, based on the limited number of data retrieved in this study. Technique sensitivity (ie, soft tissue management) may still be regarded as the main component to avoid soft tissue complications and, hence, to influence the success of bone regenerative therapy.* Int J Oral Maxillofac Implants 2018;33:41–50. doi: 10.11607/jomi.5581

Keywords: *alveolar ridge augmentation, guided bone regeneration, soft tissue complication, systematic review*

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The functional and esthetic outcomes of implant-supported prostheses are mostly related to the dimensions of the edentulous ridge. Moreover, in addition to implant width, approximately 2 mm of so-called critical buccal bone is needed in the buccal flange to avoid major physiologic bone resorption in the buccolingual plane, especially in the maxillary anterior region.[1] Therefore, in many cases, bone augmentation procedures may be required simultaneously or prior to implant placement. Several surgical techniques, such as guided bone regeneration (GBR), distraction osteogenesis, and bone block grafts have been attempted to increase bone volume and to properly withstand implant function.[1]

The GBR procedure, applying particulate autogenous bone graft or bone substitutes and an occluding membrane, has shown to be a predicable way to augment bone. This principle was initially described for periodontal tissue regeneration as a

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Biomaterials for promoting periodontal regeneration in human intrabony defects: a systematic review

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The main goals of periodontal treatment are the elimination of infection and the resolution of chronic inflammation in order to arrest disease progression and prevent its recurrence. This is manifested clinically by an absence of bleeding on probing and the presence of shallow probing pocket depths (≤4 mm) (15, 47). In contrast, the persistence of residual periodontal pockets of >5 mm following completion of active periodontal therapy is associated with an increased risk for disease progression (i.e. further loss of attachment) and tooth loss, irrespective of the presence or absence of bleeding on probing (15, 47). Increased probing depths following treatment are often related to the presence of intrabony (angular) periodontal defects, a feature of periodontitis and, in turn, intrabony defects have been shown to worsen the long-term prognosis for teeth (62). The rationale behind the treatment of intrabony defects is therefore to reduce residual probing depths to improve tooth prognosis. During the last three decades, various treatment approaches involving nonsurgical techniques, as well as conservative, resective and regenerative surgical techniques, have been employed for the treatment of intrabony defects and have achieved variable success (12, 16, 17, 37, 45, 59, 65, 69, 74, 82, 88, 97, 98).

Clinical studies have provided evidence indicating that conventional periodontal surgery, comprising various types of access flaps and/or resective techniques, may result in probing depth reduction, hard tissue fill or even the elimination of the intrabony component (37, 62, 65). However, residual pockets often persist following nonsurgical periodontal therapy or the use of access flaps, and resective techniques are associated with substantial loss of attachment and increases in soft-tissue recession (37, 62, 65, 69). Furthermore, despite the fact that such techniques may improve clinical outcomes, healing is predominantly characterized by repair (i.e. formation of a long junctional epithelium) and no, or very limited, regeneration (i.e. formation of root cementum with functionally oriented inserting periodontal ligament fibers connected to new alveolar bone) (14).

Thus, the optimal outcome of periodontal treatment is considered as the absence of bleeding on probing, the presence of shallow pockets associated with periodontal regeneration and limited/no soft-tissue recession. Since the early 1970s, a plethora of different surgical techniques, often including implantation of various types of bone grafts and/or substitutes, root surface demineralization, guided tissue regeneration, growth and differentiation factors, enamel matrix proteins or various combinations thereof, have been employed, aiming to achieve predictable periodontal regeneration (16, 17, 38, 45, 52, 74, 99). Systematic reviews of clinical trials have shown that some of these materials, when used in conjunction with surgical approaches designed to facilitate maximal preservation of soft and hard tissues, may indeed result in superior clinical outcomes in terms of probing depth reduction, clinical attach-

*Equal contribution.

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INVITED MEDICAL REVIEW

WILEY ORAL DISEASES

Stem cell-based tooth and periodontal regeneration

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Currently regeneration of tooth and periodontal damage still remains great challenge. Stem cell-based tissue engineering raised novel therapeutic strategies for tooth and periodontal repair. Stem cells for tooth and periodontal regeneration include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from the dental apical papilla (SCAPs), and stem cells from human exfoliated deciduous teeth (SHEDs), dental follicle stem cells (DFSCs), dental epithelial stem cells (DESCs), bone marrow mesenchymal stem cells (BMMSCs), adipose-derived stem cells (ADSCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). To date, substantial advances have been made in stem cell-based tooth and periodontal regeneration, including dentin–pulp, whole tooth, bioroot and periodontal regeneration. Translational investigations have been performed such as dental stem cell banking and clinical trials. In this review, we present strategies for stem cell-based tissue engineering for tooth and periodontal repair, and the translational studies.

KEYWORDS
periodontal regeneration, stem cells, tooth regeneration

1 | INTRODUCTION

The tooth is a multistructure organ composed of the highly mineralized tissues of enamel, dentin, and cementum, as well as the soft connective tissues including dental pulp and the associated periodontium. The most common diseases associated with teeth and their supporting tissues are periodontal disease, caries, and traumatic injuries. Due to its complex structure and limited self-healing capability, it is necessary to introduce external interventions to promote the biological repair of damaged dental tissue. The current restorations for tooth loss are dentures, including removable, fixed dentures, and dental implants. Resin-based composites, inlays or onlays, and artificial crowns are used for partial restoration of hard tissue defects. Routine periodontal disease treatments include basic treatment, guided tissue regeneration (GTR), and guided bone regeneration (GBR). The outcomes of these methods are limited and associated with poor clinical predictability (Needleman, Worthington,

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[Intervention Review]

Guided tissue regeneration for periodontal infra-bony defects

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ABSTRACT

Background

Conventional treatment of destructive periodontal (gum) disease arrests the disease but does not usually regain the bone support or connective tissue lost in the disease process. Guided tissue regeneration (GTR) is a surgical procedure that specifically aims to regenerate the periodontal tissues when the disease is advanced and could overcome some of the limitations of conventional therapy.

Objectives

To assess the efficacy of GTR in the treatment of periodontal infra-bony defects measured against conventional surgery (open flap debridement (OFD)) and factors affecting outcomes.

Search methods

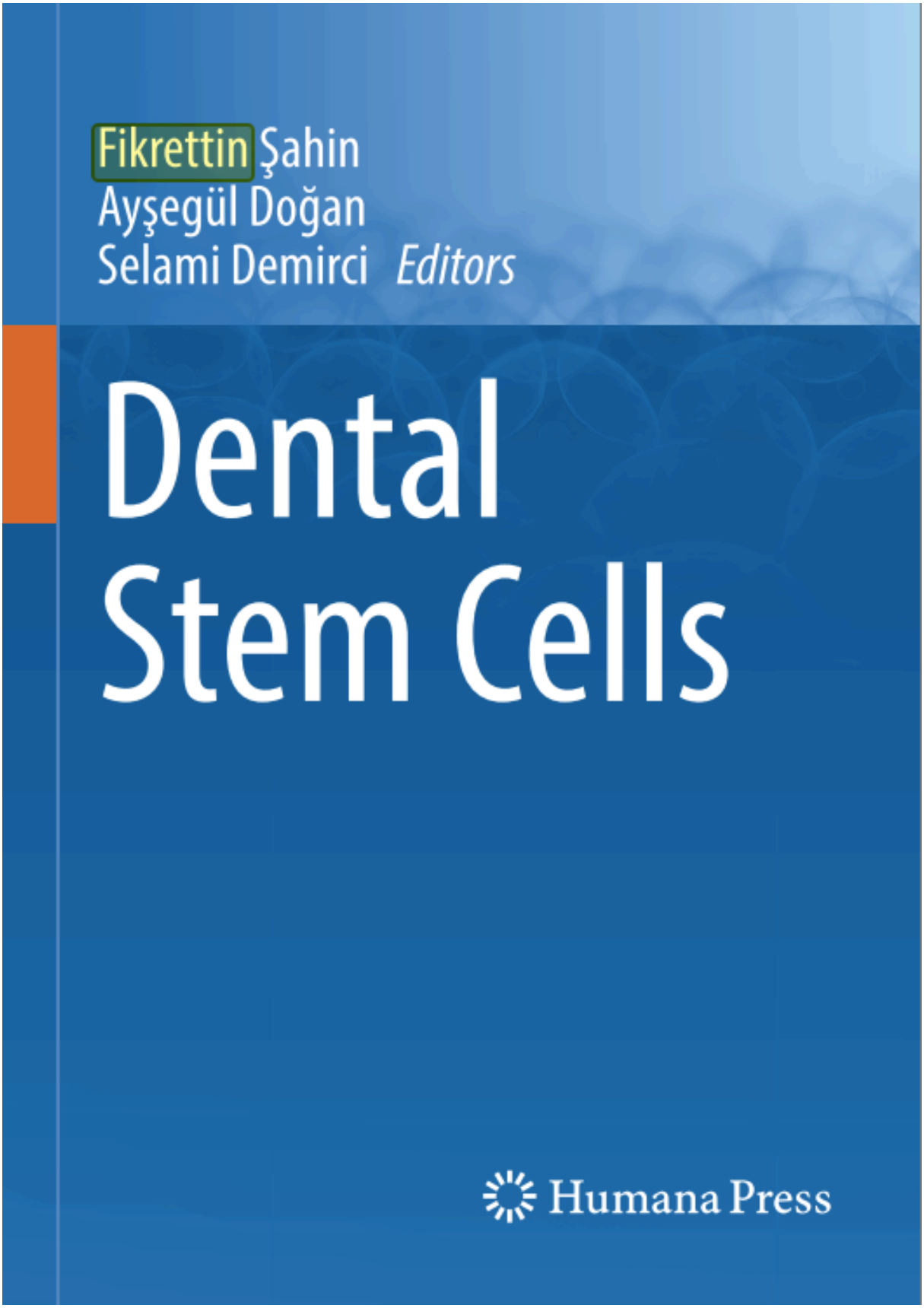
We conducted an electronic search of the Cochrane Oral Health Group Trials Register, MEDLINE and EMBASE up to April 2004. Handsearching included *Journal of Periodontology, Journal of Clinical Periodontology, Journal of Periodontal Research* and bibliographies of all relevant papers and review articles up to April 2004. In addition, we contacted experts/groups/companies involved in surgical research to find other trials or unpublished material or to clarify ambiguous or missing data and posted requests for data on two periodontal electronic discussion groups.

Selection criteria

Randomised, controlled trials (RCTs) of at least 12 months duration comparing guided tissue regeneration (with or without graft materials) with open flap debridement for the treatment of periodontal infra-bony defects. Furcation involvements and studies specifically treating aggressive periodontitis were excluded.

Data collection and analysis

Screening of possible studies and data extraction was conducted independently. The methodological quality of studies was assessed in duplicate using individual components and agreement determined by Kappa scores. Methodological quality was used in sensitivity analyses to test the robustness of the conclusions. The Cochrane Collaboration statistical guidelines were followed and the results expressed as mean differences (MD and 95% CI) for continuous outcomes and risk ratios (RR and 95% CI) for dichotomous outcomes calculated using random-effects models. Any heterogeneity was investigated. The primary outcome measure was change in clinical attachment.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Multipotent Differentiation of Human Dental Pulp Stem Cells: a Literature Review

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Abstract The advent of regenerative medicine has brought us the opportunity to regenerate, modify and restore human organs function. Stem cells, a key resource in regenerative medicine, are defined as clonogenic, self-renewing, progenitor cells that can generate into one or more specialized cell types. Stem cells have been classified into three main groups: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult/postnatal stem cells (ASCs). The present review focused the attention on ASCs, which have been identified in many perioral tissues such as dental pulp, periodontal ligament, follicle, gingival, alveolar bone and papilla. Human dental pulp stem cells (hDPSCs) are ectodermal-derived stem cells, originating from migrating neural crest cells and possess mesenchymal stem cell properties. During last decade, hDPSCs have received extensive attention in the field of tissue engineering and regenerative medicine due to their accessibility and ability to differentiate in several cell phenotypes. In this review, we have carefully described the potential of hDPSCs to differentiate into odontoblasts, osteocytes/osteoblasts, adipocytes, chondrocytes and neural cells.

Keywords Stem cells · DPSCs · Dental pulp · Tissue engineering · Regenerative medicine

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Introduction

Regenerative medicine has the potential to replace and restore tissue normal function by using the inherent ability of stem cells differentiating into specialized cell types [1]. A rich source of adult stem cells (ASCs) is located within tooth and called human dental pulp stem cells (hDPSCs). hDPSCs demonstrate high proliferative, self-renewal, and multi-lineage differentiation potential, and have been used in the fields of tissue engineering and regenerative medicine [2].

This literature review provides an overview on hDPSCs and their properties, and how recent developments have demonstrated their differentiation's potential in future regenerative medicine applications that include dental, bone, cartilage, adipose and neural regeneration.

Oral Mesenchymal Stem Cells

Stem cells (SCs) are defined as clonogenic, self-renewing, progenitor cells that can generate one or more specialized cell types [3]. Their main functions are to provide tissue development, homeostasis and reparation of damaged tissue [4].

SCs have been classified into three main groups: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and post-natal/adult stem cells (ASCs).

ASCs include: hematopoietic stem cells [5], epidermal stem cells [6], mesenchymal stem cells [7], adipose stem cells [8], neural stem cells [9], limbal stem cells [10, 11] and hepatic stem cells [12].

CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Mesenchymal Stem Cells Derived from Dental Tissues *vs.* Those from Other Sources: Their Biology and Role in Regenerative Medicine

ABSTRACT

To date, 5 different human dental stem/progenitor cells have been isolated and characterized: dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), and dental follicle progenitor cells (DFPCs). These post-natal populations have mesenchymal-stem-cell-like (MSC) qualities, including the capacity for self-renewal and multilineage differentiation potential. MSCs derived from bone marrow (BMMSCs) are capable of giving rise to various lineages of cells, such as osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic cells. The dental-tissue-derived stem cells are isolated from specialized tissue with potent capacities to differentiate into odontogenic cells. However, they also have the ability to give rise to other cell lineages similar to, but different in potency from, that of BMMSCs. This article will review the isolation and characterization of the properties of different dental MSC-like populations in comparison with those of other MSCs, such as BMMSCs. Important issues in stem cell biology, such as stem cell niche, homing, and immunoregulation, will also be discussed.

KEY WORDS: MSCs, DPSCs, SHED, SCAP, PDLSCs, DFPCs, stem cell niche, apical papilla, stem cell homing, tissue regeneration.

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INTRODUCTION

Stem cell biology has become an important field for the understanding of tissue regeneration and implementation of regenerative medicine. Since the discovery and characterization of multipotent mesenchymal stem cells (MSCs) from bone marrow (BM), MSC-like populations from other tissues have now been characterized based on the 'gold standard' criteria established for BMMSCs (Friedenstein *et al.*, 1976; Caplan, 1991; Prockop, 1997; Pittenger *et al.*, 1999; Gronthos *et al.*, 2003). Of those, MSC-like populations from adipose tissues and umbilical cord blood have been shown to be promising alternative multipotent MSC sources (Mareschi *et al.*, 2001; Zuk *et al.*, 2001). These MSCs are capable of giving rise to at least 3 cell lineages: osteogenic, chondrogenic, and adipogenic. Other lineages, such as myogenic, neurogenic, and tenogenic, may also be derived from BMMSCs. The search for MSC-like cells in specific tissues has led to the discovery of a variety of stem cells in every organ and tissue in the body in the past decades (reviewed by Baksh *et al.*, 2004; Porada *et al.*, 2006; Kolf *et al.*, 2007). Dental-tissue-derived MSC-like populations are among many other stem cells residing in specialized tissues that have been isolated and characterized. The first type of dental stem cell was isolated from the human pulp tissue and termed 'post-natal dental pulp stem cells' (DPSCs) (Gronthos *et al.*, 2000). Subsequently, 3 more types of dental-MSC-like populations were isolated and characterized: stem cells from exfoliated deciduous teeth (SHED) (Miura *et al.*, 2003), periodontal ligament stem cells (PDLSCs) (Seo *et al.*, 2004), and stem cells from apical papilla (SCAP) (Sonoyama *et al.*, 2006, 2008). Recent studies have identified a fifth dental-tissue-derived progenitor cell population, referred to as 'dental follicle precursor cells' (DFPCs) (Morsczeck *et al.*, 2005). However, the precise relationship among these different stem cell populations remains unclear.

During the characterization of these newly identified dental stem cells, certain aspects of their properties have been compared with those of BMMSCs. Dental stem cells display multidifferentiation potential, with the capacity to give rise to at least 3 distinct cell lineages: osteo/odontogenic, adipogenic, and neurogenic. Differences have been noted between the dental stem cell populations and BMMSCs, where dental stem cells appear to be more committed to odontogenic rather than osteogenic development. To date, dental-tissue-derived stem/progenitor cells have been used for tissue-engineering studies in large animals to assess their potential in pre-clinical

STEM CELLS
TISSUE-SPECIFIC STEM CELLS

Periodontal Ligament Stem Cells Regulate B Lymphocyte Function Via Programmed Cell Death Protein 1

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Key Words. Immunoregulation • Mesenchymal stem cells • Periodontal ligament stem cells • Periodontitis • B lymphocyte • Humoral immunity • Programmed cell death protein 1

ABSTRACT

Periodontal ligament stem cells (PDLSCs) have provided novel cell sources for tooth and periodontal tissue regeneration. Allogeneic PDLSCs can reconstruct periodontal ligament tissue that has been damaged by periodontal diseases and regulate T-cell immunity. However, the effect of PDLSCs on B cells remains unknown. Here, we treated periodontitis in a miniature pig model using allogeneic PDLSCs and showed a reduction in humoral immunity in the animals. When cocultured with normal B cells, human PDLSCs (hPDLSCs) had similar effects as bone marrow mesenchymal stem cells in suppressing B cell proliferation, differentiation, and migration, while intriguingly, hPDLSCs increased B cell viability by secreting interleukin-6. Mechanistically, hPDLSCs suppressed B cell activation through cell-to-cell contact mostly mediated by programmed cell death protein 1 and programmed cell death 1 ligand 1. Our data revealed a previously unrecognized function of PDLSCs in regulating humoral immune responses, which may represent a novel therapeutic strategy for immune-related disorders. STEM CELLS *2013;31:1371–1382*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Adult stem cells exist in many tissues, including bone marrow, skin, adipose tissue, tendon, lung, heart, liver, placenta, and umbilical cord blood [1–9]. Due to their low immunogenicity and immunoregulatory function, mesenchymal stem cells (MSCs) play a key role in tissue regeneration and have been used in clinical trials in therapy for severe refractory diseases [10–12].

Periodontitis is one of the most widespread chronic infectious diseases in humans, which is the most common cause for tooth loss in adults. Several factors are known to be involved in the occurrence of periodontitis [13, 14]. Our previous study showed that periodontal ligament stem cells (PDLSCs) from periodontitis tissue had impaired immunomodulatory function, which may lead to an imbalanced immune response and the acceleration of osteoclastogenesis and inflammation related bone loss [15]. PDLSCs and other tooth-related stem cells have provided new prospects and potential therapeutic cell sources for tooth regeneration and the reconstruction of periodontal ligament tissue damaged by periodontal diseases [16–18]. However, limited cell sources of autologous dental stem cell populations impede the potential of clinical application.

Allogeneic dental stem cells significantly enlarged the source of seed cells for teeth and periodontal tissue regeneration and reconstruction development. We demonstrated previously that allogeneic PDLSCs exhibited immunosuppressive activities on activated T-cells in vitro [18]. In vivo allogeneic PDLSCs can regenerate and reconstruct periodontal ligament damaged by periodontal diseases and inactivate T-cell immunity. However, the effects of PDLSCs on B cells are unknown. Here, we investigated whether allogeneic PDLSCs could affect B-cell immune responses in vitro and in vivo. We also studied human PDLSC (hPDLSC)-mediated regulation of human B cells. We showed that allogeneic PDLSCs failed to activate humoral immunity in vivo in miniature pigs. We also demonstrated that hPDLSCs inhibit human B cell proliferation, differentiation, and chemotactic behavior. hPDLSCs secreted interleukin (IL)-6 and enhanced B-cell survival. The immunoregulatory function of hPDLSCs in human B cells was achieved by programmed death-1 (PD-1) and its

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Review

Cells Tissues Organs

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Stem Cells from Human Exfoliated Deciduous Teeth: A Growing Literature

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Key Words
Dental tissue · Mesenchymal stem cells · Stem cells · Teeth

Abstract
Adult stem cells research has been considered the most advanced sort of medical-scientific research, particularly stem cells from human exfoliated deciduous teeth (SHED), which represent an immature stem cell population. The purpose of this review is to describe the current knowledge concerning SHED from full-text scientific publications from 2003 to 2015, available in English language and based on the keyword and/or abbreviations 'stem cells from human exfoliated deciduous teeth (SHED)', and individually presented as to the properties of SHED, immunomodulatory properties of SHED and stem cell banking. In summary, these cell populations are easily accessible by noninvasive procedures and can be isolated, cultured and expanded in vitro, successfully differentiated in vitro and in vivo into odontoblasts, osteoblasts, chondrocytes, adipocytes and neural cells, and present low immune reactions or rejection following SHED transplantation. Furthermore, SHED are able to remain undifferentiated and stable after long-term cryopreservation. In conclusion, the high proliferative capacity, easy access, multilineage differentiation capacity, noninvasiveness and few ethical concerns make stem cells from human exfoliated deciduous teeth the most valuable source of stem cells for tissue engineering and cell-based regenerative medicine therapies.

Introduction

The discovery of stem cells was an important progress in regenerative medicine and opened a new era of experimentation, with potential in the therapy of various diseases. The stem cells are clonogenic and capable of self-renewal, multilineage differentiation and stemness.

According to the ability and potency to differentiate into different cellular types, three types of stem cells have been established: (1) totipotent stem cells, which are able to develop into an entire organism; (2) pluripotent stem cells, known as embryonic stem cells, which under induced conditions are capable of differentiating into all types of tissue, and (3) multipotent stem cells, which are postnatal stem cells or adult stem cells with capability of multilineage differentiation [Wagers and Weissman, 2004].

KARGER

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REVIEW

Dental stem cell and dental tissue regeneration

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Abstract **The teeth are highly differentiated chewing organs formed by the development of tooth germ tissue located in the jaw and consist of the enamel, dentin, cementum, pulp, and periodontal tissue. Moreover, the teeth have a complicated regulatory mechanism, special histologic origin, diverse structure, and important function in mastication, articulation, and aesthetics. These characteristics, to a certain extent, greatly complicate the research in tooth regeneration. Recently, new ideas for tooth and tissue regeneration have begun to appear with rapid developments in the theories and technologies in tissue engineering. Numerous types of stem cells have been isolated from dental tissue, such as dental pulp stem cells (DPSCs), stem cells isolated from human pulp of exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs), and dental follicle cells (DFCs). All these cells can regenerate the tissue of tooth. This review outlines the cell types and strategies of stem cell therapy applied in tooth regeneration, in order to provide theoretical basis for clinical treatments.**

Keywords stem cells; pulp regeneration; periodontal regeneration

Introduction

Stem cells are primitive cells with self-replicating and multi-directional differentiation potentials. Moreover, they can be differentiated into various functional cells or tissues and organs under certain conditions, and are thus known as "universal cells." Stem cell therapy is the use of the multi-directional differentiation characteristics of stem cells in order to repair diseased cells or reconstruct normal functioning of cells and tissues [1]. Therefore, stem cell therapy has provided new hope for tissue and organ regeneration.

In recent years, new ideas for tooth and tissue regeneration have been proposed secondary to the rapid developments in theories and technologies in tissue engineering. Various types of stem cells and new biological methods such as those that use bioactive factors, have been widely applied in tooth regeneration research [2]. The teeth are highly differentiated chewing organs formed by the development of tooth germ tissue located in the jaw and consist of the enamel, dentin, cementum, pulp, and periodontal tissue. Moreover, the teeth have a complicated regulatory mechanism, special histologic origin, diverse structure, and important function. These characteristics, to a certain extent, greatly complicate the research in tooth regeneration.

Feasible availability is one of the superior properties of dental stem cells. Dental stem cells can be easily obtained from premolar and wisdom teeth and are thus extracted for orthodontics use; moreover, dental stem cells are increasingly becoming the source for regenerative medicine research. Healthy dental tissues contain large amounts of normal stem cells that are needed to maintain normal function, whereas inflamed or traumatized tissues have a low amount of robust stem cells, which leads to failure in tissue repair [3,4]. Thus, *ex vitro* expansion/manipulation of stem cells is seen to be an important source when it comes to supplementing host cells and promoting tissue

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Utility of PDL progenitors for *in vivo* tissue regeneration: a report of 3 cases

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Abstract

Objective—Periodontal disease is an inflammatory disorder with widespread morbidities involving both oral and systemic health. The primary goal of periodontal treatment is the regeneration of the lost or diseased periodontium. In this study, we retrospectively examined feasibility and safety of reconstructing the periodontal intrabony defects with autologous periodontal ligament progenitor (PDLP) implantation in three patients.

Materials and Methods—In this retrospective pilot study, we treated 16 teeth with at least one deep intrabony defect of probing depth (PD) ≥ 6 mm with PDLP transplantation and evaluated clinical outcome measures in terms of probing depth, gingival recession and attachment gain for a duration of 32–72 months. Furthermore, we compare PDLPs with standard PDL stem cells (PDLSCs) and confirmed that PDLPs possessed progenitor characters.

Results—Clinical examination indicated that transplantation of PDLPs may provide therapeutic benefit for the periodontal defects. All treated patients showed no adverse effects during the entire course of follow up. We also found that PDLPs were analogous to PDLSCs in terms of high proliferation, expression of mesenchymal surface molecules, multipotent differentiation, and *in vivo* tissue regain. However, PDLPs failed to express scleraxis, a marker of tendon, as seen in PDLSCs.

Conclusions—This study demonstrated clinical and experimental evidences supporting a potential efficacy and safety of utilizing autologous PDL cells in the treatment of human periodontitis.

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Heterogeneous Dental Follicle Cells and the Regeneration of Complex Periodontal Tissues

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Dental follicle cells (DFCs) are a heterogeneous population that exhibit a variety of phenotypes. However, it remains unclear whether DFCs can maintain stem cell characteristics, or mediate tissue-regeneration to form single or complex tissues in the periodontium, after long-term culturing. Therefore, DFCs were isolated from human impacted molars (HIM-DFCs), passaged >30 times, and then evaluated for their heterogeneity and multipotential differentiation. Morphology, proliferation, epitope profile, and mineralization characteristics of clones derived from single HIM-DFCs *in vitro* were also assayed. HIM-DFCs (passage #30) were found to be positive for the heterogeneous markers, Notch-1, stro-1, alkaline phosphomonoesterase (ALP), type I collagen (COL-I), type III collagen (COL-III), and osteocalcine. Moreover, passage #30 of the HDF1, 2, and 3 subclone classes identified in this study were found to express high levels of the mesenchymal stem cells markers, CD146 and Stro1. HDF3 subclones were also associated with the strongest ALP staining detected, and strongly expressed osteoblast and cementoblast markers, including *COL-I, COL-III,* bone sialoprotein (*BSP*), and *Runx2*. In contrast, HDF1 subclone analyzed strongly expressed *COL-I* and *COL-III*, yet weakly expressed *BSP* and *Runx2*. The HDF2 subclone was associated with the strongest proliferative capacity. To evaluate differentiation characteristics *in vivo*, these various cell populations were combined with ceramic bovine bone and implanted into subcutaneous pockets of nude mice. The 30th passage of subclone HDF1 and 3 were observed to contribute to fiber collagens and the mineralized matrix present, respectively, whereas HDF2 subclones were found to have a minimal role in these formations. The formation of a cementum-periodontal ligament (PDL) complex was observed 6 weeks after HIM-DFCs (passage #30) were implanted *in vivo*, thus suggesting that these cells maintain stem cell characteristics. Therefore, subclone HDF1-3 may be related to the differentiation of fibroblasts in the PDL, undifferentiated cells, and osteoblasts and cementoblasts, respectively. Overall, this study is the first to amplify HIM-DFCs and associated subclones with the goal of reconstructing complex or single periodontium. Moreover, our results demonstrate the potential for this treatment approach to address periodontal defects that result from periodontitis, or for the regeneration of teeth.

Introduction

Periodontitis is a disease that can threaten an individual's health, and unfortunately, is frequently reported. Development of periodontitis can be associated with varying degrees of tissue damage, or tooth loss, which can involve a single tissue, such as the cementum, periodontal ligament (PDL), or alveolar bone (AB), or complex tissues including the PDL-cementum complex, the PDL-AB complex, or the AB-PDL-cementum complex. The optimal treatment strategy for these conditions is to restore the affected tissues. Therefore, we investigated whether the seeding of dental follicle cells (DFCs) would provide a heterogeneous population of cells that could be induced to generate an engineered periodontal tissue (EPT).

The dental follicle contains embryogenic tissues that surround the tooth germ during tooth genesis and development. DFCs have been characterized to be a heterogeneous cell population,[1] which may contain precursors of the cellular components involved in periodontal development. Correspondingly,

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Mesenchymal Stem Cells Derived from Human Gingiva Are Capable of Immunomodulatory Functions and Ameliorate Inflammation-Related Tissue Destruction in Experimental Colitis[1]

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Abstract

Aside from the well-established self-renewal and multipotent differentiation properties, mesenchymal stem cells exhibit both immunomodulatory and anti-inflammatory roles in several experimental autoimmune and inflammatory diseases. In this study, we isolated a new population of stem cells from human gingiva, a tissue source easily accessible from the oral cavity, namely, gingiva-derived mesenchymal stem cells (GMSCs), which exhibited clonogenicity, self-renewal, and multipotent differentiation capacities. Most importantly, GMSCs were capable of immunomodulatory functions, specifically suppressed peripheral blood lymphocyte proliferation, induced expression of a wide panel of immunosuppressive factors including IL-10, IDO, inducible NO synthase (iNOS), and cyclooxygenase 2 (COX-2) in response to the inflammatory cytokine, IFN-γ. Cell-based therapy using systemic infusion of GMSCs in experimental colitis significantly ameliorated both clinical and histopathological severity of the colonic inflammation, restored the injured gastrointestinal mucosal tissues, reversed diarrhea and weight loss, and suppressed the overall disease activity in mice. The therapeutic effect of GMSCs was mediated, in part, by the suppression of inflammatory infiltrates and inflammatory cytokines/mediators and the increased infiltration of regulatory T cells and the expression of anti-inflammatory cytokine IL-10 at the colonic sites. Taken together, GMSCs can function as an immunomodulatory and anti-inflammatory component of the immune system in vivo and is a promising cell source for cell-based treatment in experimental inflammatory diseases.

Mesenchymal stem cells (MSCs)[3] have the capacity to self-renew and differentiate into different cell lineages, including mesodermal, endodermal, and ectodermal cells (1,2).

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Disclosures
The authors have no financial conflict of interest.

RESEARCH ARTICLE

Isolation and characterization of mesenchymal stem cell-like cells from healthy and inflamed gingival tissue: potential use for clinical therapy

Aim: Postnatal mesenchymal stem cell (MSC)-like cells have previously been isolated and *ex vivo*-expanded from healthy gingival tissues. The aim of this research was to isolate and characterize MSC-like cells from inflamed gingival tissues and determine whether they retain the characteristics of MSC-like cells from healthy gingival tissues. **Materials & methods:** Fifteen clonal lines of MSC-like cells from three healthy gingival tissues (GMSC-H) and fifteen from three inflamed gingival tissues (GMSC-I) were generated. Bulk-cultured cell lines from healthy and inflamed gingival tissues were also established. *In vitro* and *in vivo* characterization studies of GMSC-Is were performed relative to GMSC-Hs. **Results:** The incidence of donogenic colony forming units-fibroblast was comparable between healthy and inflamed gingival tissues. GMSC-H and GMSC-I clones expressed MSC-associated markers CD44, CD73, CD90, CD105 and CD166. While the population doubling capacity of GMSC-Is was reduced compared with GMSC-Hs, both populations displayed a similar capacity to undergo osteogenic, adipogenic and chondrogenic differentiation *in vitro*. Following subcutaneous implantation in NOD/SCID mice, both GMSC-Hs and GMSC-Is formed dense connective tissue-like structures *in vivo* resembling natural gingival tissue. **Conclusion:** MSC-like populations exist within inflamed gingival tissue that are functionally equivalent to MSC-like cells derived from healthy gingival tissue. Given the relative abundance of inflamed gingival tissue and ease of accessibility, MSC-like cells from inflamed gingival tissues represent a newly identified population of postnatal stem cells with immense potential in tissue engineering applications.

KEYWORDS: differentiation • gingival • inflammation • mesenchymal stem cells • tissue engineering

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Postnatal mesenchymal stem cells (MSCs) represent a population of nonhematopoietic fibroblast-like cells that display the capacity to self-renew and differentiate into multiple lineages, including osteoblasts, adipocytes and chondrocytes [1,2]. While traditionally isolated from bone marrow and other sources, including adipose tissue and umbilical cord blood, MSC-like cell populations have also been isolated from mature and developing dental tissues, including dental pulp, periodontal ligament, exfoliated deciduous teeth, dental follicle and root apical papilla [3–10]. Given the innate capacity of dental-derived MSC-like cells to ectopically generate structures resembling the tissues from which they are derived *in vivo*, these progenitor cell populations represent promising candidates for oral tissue regeneration [6–8]. However, the utilization of dental tissue-derived MSC-like cells for clinical application is limited by the requirement for tooth extraction.

Gingival tissue overlying the alveolar bone of tooth sockets plays an essential role in acting as a mucosal barrier against constant mechanical and bacterial insults. Interestingly, gingival tissue exhibits fetal-like scarless wound healing properties in contrast to scar formation commonly observed in damaged extra-oral tissues [11,12]. Zhang *et al.* first isolated a population of progenitor cells within gingival tissue, termed gingiva-derived MSCs (GMSCs), which formed clonogenic colonies, expressed a typical MSC surface marker profile and possessed the ability to differentiate into multiple mesodermal lineages *in vitro* [13]. Notably, single colony-derived GMSCs demonstrated the capacity for self-renewal and formation of connective tissue-like structures *in vivo* [13]. Interestingly, GMSCs also appear to possess immunomodulatory properties in terms of suppression of lymphocyte proliferation and have been shown to be effective in treating various inflammatory and autoimmune diseases in experimental models [13,14]. Given the relative accessibility and availability of human gingival tissue following some dental procedures and that subsequent healing usually occurs in a short time period following surgery [15], the clinical use of GMSCs for tissue regeneration and repair represents an attractive therapeutic option. However, obtaining healthy gingival tissues may be challenging at a specific time of need.

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Gingival Fibroblasts as a Promising Source of Induced Pluripotent Stem Cells

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Abstract

Background: Induced pluripotent stem (iPS) cells efficiently generated from accessible tissues have the potential for clinical applications. Oral gingiva, which is often resected during general dental treatments and treated as biomedical waste, is an easily obtainable tissue, and cells can be isolated from patients with minimal discomfort.

Methodology/Principal Findings: We herein demonstrate iPS cell generation from adult wild-type mouse gingival fibroblasts (GFs) via introduction of four factors (Oct3/4, Sox2, Klf4 and c-Myc; GF-iPS-4F cells) or three factors (the same as GF-iPS-4F cells, but without the c-Myc oncogene; GF-iPS-3F cells) without drug selection. iPS cells were also generated from primary human gingival fibroblasts via four-factor transduction. These cells exhibited the morphology and growth properties of embryonic stem (ES) cells and expressed ES cell marker genes, with a decreased CpG methylation ratio in promoter regions of Nanog and Oct3/4. Additionally, teratoma formation assays showed ES cell-like derivation of cells and tissues representative of all three germ layers. In comparison to mouse GF-iPS-4F cells, GF-iPS-3F cells showed consistently more ES cell-like characteristics in terms of DNA methylation status and gene expression, although the reprogramming process was substantially delayed and the overall efficiency was also reduced. When transplanted into blastocysts, GF-iPS-3F cells gave rise to chimeras and contributed to the development of the germline. Notably, the four-factor reprogramming efficiency of mouse GFs was more than 7-fold higher than that of fibroblasts from tail-tips, possibly because of their high proliferative capacity.

Conclusions/Significance: These results suggest that GFs from the easily obtainable gingival tissues can be readily reprogrammed into iPS cells, thus making them a promising cell source for investigating the basis of cellular reprogramming and pluripotency for future clinical applications. In addition, high-quality iPS cells were generated from mouse GFs without Myc transduction or a specific system for reprogrammed cell selection.

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Introduction

Direct reprogramming of somatic cells into induced pluripotent stem (iPS) cells by forced expression of a small number of defined factors (e.g., Oct3/4, Sox2, Klf4 and c-Myc) has great potential for tissue-specific regenerative therapies, avoiding ethical issues surrounding the use of embryonic stem (ES) cells and problems with rejection following implantation of non-autologous cells. The iPS cells have been generated from a variety of mammalian species including mice [1], monkeys [2], dogs [3], pigs [4] and humans [5–8]. Mouse iPS cells have been generated from cells of all three embryonic germ layers, including mesodermal fibroblasts [1] and B lymphocytes [9], endodermal hepatocytes [10], gastric epithelial cells [10] and pancreatic cells [11], and ectodermal keratinocytes [12].

The reprogramming process appears to be highly inefficient and is likely affected by many factors, including the age, type and origin of the cells used. Recently, a "stochastic model" predicted that most or all cells are competent for reprogramming [13]. However, the kinetics of reprogramming appear to vary when target populations from different tissues are used. Mouse hepatocytes and gastric epithelial cells appear to be more easily reprogrammed and require less retroviral integration than fibroblasts [10]. Dermal papilla cells, which endogenously express high levels of Sox2 and c-Myc, have been reported to be reprogrammed more efficiently than skin and embryonic fibroblasts [14]. Although the mechanisms underlying differences in reprogramming efficiency are not yet clear, some cell types might be more easily reprogrammed using specific exogenous factors than others. Importantly, the use of cell types with a high reprogramming efficiency could reduce the number of transduced factors needed, decreasing the chance of retroviral insertional mutagenesis and increasing the likelihood of ultimately replacing the remaining factors with small molecules [15]. For future clinical application, it is therefore crucial to identify cell types that can be

RAPID COMMUNICATION
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Stem-cell-based Tissue Engineering of Murine Teeth

ABSTRACT

Teeth develop from reciprocal interactions between mesenchyme cells and epithelium, where the epithelium provides the instructive information for initiation. Based on these initial tissue interactions, we have replaced the mesenchyme cells with mesenchyme created by aggregation of cultured non-dental stem cells in mice. Recombinations between non-dental cell-derived mesenchyme and embryonic oral epithelium stimulate an odontogenic response in the stem cells. Embryonic stem cells, neural stem cells, and adult bone-marrow-derived cells all responded by expressing odontogenic genes. Transfer of recombinations into adult renal capsules resulted in the development of tooth structures and associated bone. Moreover, transfer of embryonic tooth primordia into the adult jaw resulted in development of tooth structures, showing that an embryonic primordium can develop in its adult environment. These results thus provide a significant advance toward the creation of artificial embryonic tooth primordia from cultured cells that can be used to replace missing teeth following transplantation into the adult mouth.

KEY WORDS: tooth development, bone-marrow-derived cells, stem cells, tissue engineering.

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INTRODUCTION

The application of stem-cell-based tissue-engineering approaches to create organs and tissues for transplantation requires an understanding and manipulation of the developmental processes that direct organ/tissue formation in the embryo, a source of cells with multipotential that can be easily cultured, and an ability of an organ rudiment to form the complete organ in the adult environment (Bianco and Robey, 2001). In common with most organs, teeth develop from interactions between epithelial cells (oral epithelium) and mesenchyme cells (cranial neural-crest-derived ectomesenchyme) (Thesleff *et al.*, 1995; Thesleff and Sharpe, 1997). Evidence accumulated from a variety of molecular and cellular studies has established that the embryonic oral epithelium provides the instructive signals for tooth initiation and shape determination. These signals principally consist of spatially restricted secreted protein ligands that are received by the ectomesenchyme cells which are then primed to become odontogenic and in turn act as a source of reciprocal signals back to the epithelium. In the early stages of jaw development (up to E10 in mice), all ectomesenchyme cells appear able to respond to signals such as FGF8 and BMP4 from the oral epithelium (Ferguson *et al.*, 2000). These observations, together with the ability of E10 embryonic oral epithelium to direct odontogenesis when recombined with non-dental ectomesenchyme, such as that from the second pharyngeal arch, suggest that ectomesenchyme cells are plastic in their responses to oral epithelial signals, and thus cranial neural crest cells do not contain an inherent odontogenic pre-specification (Mina and Kollar, 1987; Lumsden, 1988). These properties, together with the multipotentiality of cranial neural crest cells, prompted us to investigate the ability of cultured non-dental cells to replace ectomesenchyme cells and contribute to tooth formation.

MATERIALS & METHODS

Culture of Non-dental Cells

Feeder-independent mouse embryonic stem cells (E14.2) were cultured in D-MEM with 10^3 U/mL of leukemia inhibitory factor, buffalo rat liver cell-conditional medium, 200 mM L-glutamine, non-essential amino acid, and 2-mercaptoethanol. Medium was changed every day, and ES cells were passaged every 2-3 days. Duplicate flasks of the cells were used to generate a mouse gene knock-out that has subsequently resulted in two lines of mice with full germline transmission (unpublished).

Neural stem cells were isolated from E14 embryo spinal cords at the level of the upper limb to the lower cervical region. The cord itself was carefully dissected free from any other tissue and membrane to reveal nothing but naked spinal cord. The cord was then dissociated into single cells by the use of trypsin and flame-narrow pipettes and plated at 200,000 *per* T-75 on 10 μg/mL poly-ornithine and 10 μg/mL laminin in serum-free medium (DMEM/F12)

STEM CELLS TRANSLATIONAL MEDICINE

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Periodontal Tissue Regeneration Using Syngeneic Adipose-Derived Stromal Cells in a Mouse Model

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Key Words. Mesenchymal stromal cells • Mesenchymal stem cell transplantation • Mice • Periodontitis • Regenerative medicine • Subcutaneous fat

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ABSTRACT

Current treatment of periodontitis is still associated with a high degree of variability in clinical outcomes. Recent advances in regenerative medicine by mesenchymal cells, including adipose stromal cells (ASC) have paved the way to improved periodontal regeneration (PD) but little is known about the biological processes involved. Here, we aimed to use syngeneic ASCs for periodontal regeneration in a new, relevant, bacteria-induced periodontitis model in mice. Periodontal defects were induced in female C57BL6/J mice by oral gavage with periodontal pathogens. We grafted 2×10^5 syngeneic mouse ASCs expressing green fluorescent protein (GFP) (GFP+/ASC) within a collagen vehicle in the lingual part of the first lower molar periodontium (experimental) while carrier alone was implanted in the contralateral side (control). Animals were sacrificed 0, 1, 6, and 12 weeks after treatment by GFP+/ASC or vehicle graft, and microscopic examination, immunofluorescence, and innovative bio-informatics histomorphometry methods were used to reveal deep periodontium changes. From 1 to 6 weeks after surgery, GFP+ cells were identified in the periodontal ligament (PDL), in experimental sites only. After 12 weeks, cementum regeneration, the organization of PDL fibers, the number of PD vessels, and bone morphogenetic protein-2 and osteopontin expression were greater in experimental sites than in controls. Specific stromal cell subsets were recruited in the newly formed tissue in ASC-implanted periodontium only. These data suggest that ASC grafting in diseased deep periodontium, relevant to human pathology, induces a significant improvement of the PDL microenvironment, leading to a recovery of tooth-supporting tissue homeostasis. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:656–665*

SIGNIFICANCE STATEMENT

Human periodontitis is a chronic, highly prevalent infectious disease characterized by the loss of both soft and hard tissues supporting the teeth. Current available treatments are insufficient, associated with a high degree of variability in clinical outcomes. The data in this study suggest that adipose-derived mesenchymal stromal/stem cell (ASC) grafting in diseased deep periodontium, relevant to human pathology, promoted regeneration of deep periodontium, both in quantity and in quality, in comparison with controls. Even if mechanisms underlying periodontal regeneration by exogenous mesenchymal stromal cells are yet to be understood, this study brought to light new data regarding periodontal pocket regeneration induced by ASCs in mice.

INTRODUCTION

Periodontitis is a chronic immuno-infectious disease, characterized by loss of the tissues supporting the teeth, and leading to or aggravating systemic disorders such as diabetes, polyarthritis, or atherosclerosis [1]. The defects result from a local homeostasis disruption caused by both the virulence of a periodontal pathogenic microflora [2] and an inappropriate immune response [3, 4]. From a pathophysiology point of view [3, 4], the destruction of deep periodontium tissues (i.e., root cementum, periodontal ligament [PDL], and alveolar bone) induces the formation of crevices called "periodontal pockets" between the tooth root and its bony socket [5], leading to tooth loss.

Periodontal regeneration aims to restore both the architecture and function of tooth supporting tissues through the recruitment and activation of endogenous progenitors, especially those expressing CD146 markers [5], leading to renewal of the connective attachment underlying the new junctional epithelium. The restitution of dense connective fibers of PDL, anchored between the newly formed alveolar bone and root cementum, is critical for the long-term prognosis

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A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration

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ABSTRACT

In this study, several *in vivo* and *in vitro* comparisons were performed to test the possibility of using adipose-derived stem cells (ADSCs), a more convenient cell source than dental pulp stem cells (DPSCs), in tooth regeneration. Using an efficient, non-engineering implantation method, we first demonstrated that both implants of ADSCs and DPSCs were able to grow self-assembled new teeth in adult rabbit extraction sockets with high success rate. The stem cells were necessary because the implants grew no tooth without them. A stepwise comparison showed that the regenerated teeth from these two types of adult stem cells were living with nerves and vascular system and remarkably similar to a normal tooth in many details. Further strictly controlled, side-by-side comparisons between the two types of stem cells also showed that the expression patterns of gene markers and the broad differentiation potentials induced by specific methods *in vitro* were very similar. Although a few differences were found, they did not affect the tested tooth regeneration *in vivo* or differentiation *in vitro*. Furthermore, rabbit ADSCs had a higher growth rate and a better senescence resistance in culture. All these findings suggest that ADSCs, one of the richest adult stem cells in mammals, are very similar and useful as DPSCs for regenerative dentistry.

1. Introduction

Endodontics, periodontics, and prosthodontics in dentistry are all entering a new era of using stem cells to repair and even to regenerate bio-teeth or natural teeth [1,2]. Several types of dental stem cells were isolated and studied for this purpose [3]. Among them, DPSCs, a type of mesenchymal stem cell (MSC), have been studied the most on their odontoblast-like features and differentiation potentials to become dental tissues, but the goal to regenerate a self-organized whole living tooth has never been attained yet [4–9]. In fact, owing to the complexity of organogenesis in higher animals, none of the biomedical attempts, except reconstruction of the tooth developmental steps [10,11], has ever led to the unstructured production of a living tooth in adult mammals from any type of adult stem cells, including dental stem cells. In addition to the morphogenic intricacy required to develop a whole tooth in regenerative dentistry, obtaining autologous cell sources to treat patients with missing or decayed teeth will be an arduous struggle. The cell source for the engineered teeth by reconstitution of development process was from embryonic tooth germ which is unlikely to obtain for a patient in clinics [11]. Even if the DPSCs could produce a new tooth, it would be very inconvenient to acquire them from a patient since the isolated cells are better from a patient's healthy pulp. Hence, the idea of using MSCs from other tissues, including skin dermis, hair follicle, bone marrow, and adipose tissue, in regenerative dentistry has emerged [12–15]. These types of MSCs which are rich in our body can be extracted easily at any time without costly cryopreservation. ADSCs are an especially great MSC source for this purpose because of the less invasive surgery required to obtain them, their growth rate, and differentiation potentials [16,17].

To test if ADSCs can replace DPSCs in regenerative dentistry, we executed two strategic plans in this study: 1) to observe whether implants of both types of stem cells can generate new teeth *in vivo* and 2) to compare the implants, their cultural growth and senescence,

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Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

INTRODUCTION

Embryonic stem (ES) cells, which are derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (Evans and Kaufman, 1981; Martin, 1981). Human ES cells might be used to treat a host of diseases, such as Parkinson's disease, spinal cord injury, and diabetes (Thomson et al., 1998). However, there are ethical difficulties regarding the use of human embryos, as well as the problem of tissue rejection following transplantation in patients. One way to circumvent these issues is the generation of pluripotent cells directly from the patients' own cells.

Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997) or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), *c-myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and *β-catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

RESULTS

We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity (see Table S1 in the Supplemental Data available with this article online). For β-catenin, c-Myc, and Stat3, we used active forms, S33Y-β-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively. Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2ΔSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

Clinical Review

Induced Pluripotent Stem Cells: A New Frontier for Stem Cells in Dentistry

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Abstract

Induced pluripotent stem cells (iPSCs) are the newest member of a growing list of stem cell populations that hold great potential for use in cell-based treatment approaches in the dental field. This review summarizes the dental tissues that have successfully been utilized to generate iPSC lines, as well as the potential uses of iPSCs for tissue regeneration in different dental applications. While iPSCs display great promise in a number of dental applications, there are safety concerns with these cells that need to be addressed before they can be used in clinical settings. This review outlines some of the apprehensions to the use of iPSCs clinically, and it details approaches that are being employed to ensure the safety and efficacy of these cells. One of the major approaches being investigated is the differentiation of iPSCs prior to use in patients. iPSCs have successfully been differentiated into a wide range of cells and tissue types. This review focuses on 2 differentiation approaches—the differentiation of iPSCs into mesenchymal stem cells and the differentiation of iPSCs into osteoprogenitor cells. Both these resulting populations of cells are particularly relevant to the dental field.

Keywords: cell differentiation, regenerative medicine, tissue engineering, mesenchymal stem cells, bone regeneration, cell lineage

Induced Pluripotent Stem Cells

In 2006 it was discovered that stem cells could be generated from adult somatic cells through a process of cellular reprogramming (Takahashi and Yamanaka 2006). Stem cells generated by this new technology were termed *induced pluripotent stem cells* (iPSCs). Since their discovery, there has been substantial interest in iPSCs, as this technology facilitates the generation of adult human pluripotent stem cells without the need for human embryos, thereby bypassing a number of the ethical and legal concerns that have hindered embryonic stem cell research to date (Takahashi and Yamanaka 2006; Maherali et al. 2007; Okita et al. 2007; Takahashi et al. 2007; Wernig et al. 2007). iPSCs appear to have a number of advantages over other dental-derived stem cell populations, such as periodontal ligament (PDL) and dental pulp. Specifically, iPSCs can be generated from readily accessible tissue sources, including oral mucosa (Miyoshi et al. 2010) and gingival tissue (Egusa et al. 2010). More important, iPSCs are highly proliferative, making it possible to obtain the large numbers of stem cells that would be required for use in regenerative therapies in the clinic.

Limitations of iPSCs

Before we outline the potential of iPSCs in dentistry, it is important to note that there are significant safety concerns regarding iPSCs that need to be addressed before they can be considered for use in mainstream treatment approaches in dentistry. The major drawbacks of iPSCs include their genomic instability and their propensity to form tumors in vivo (Ben-David and Benvenisty 2011; Gore et al. 2011). While there are shortcomings associated with iPSCs, the potential that iPSCs have demonstrated in the treatment of multiple disorders demands further research to investigate and minimize the associated therapeutic risks.

The use of viral integrating vectors in the generation of iPSCs is a contributing factor to genomic instability and tumorigenic potential of iPSCs. Consequently, various groups have attempted to utilize nonintegrating vectors to reduce the genomic instability of iPSCs. Zou et al. (2012) successfully utilized a single lentiviral stem cell cassette to generate iPSCs from human stem cells of apical papilla. Stem cell cassette is a single lentiviral cassette flanked by a lox-p site, which allows for its controlled excision with cre-recombinase (Somers et al. 2010). The ability to remove the transgene/vector from the

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iPS Cells Reprogrammed From Human Mesenchymal-Like Stem/Progenitor Cells of Dental Tissue Origin

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Generation of induced pluripotent stem (iPS) cells holds a great promise for regenerative medicine and other aspects of clinical applications. Many types of cells have been successfully reprogrammed into iPS cells in the mouse system; however, reprogramming human cells have been more difficult. To date, human dermal fibroblasts are the most accessible and feasible cell source for iPS generation. Dental tissues derived from ectomesenchyme harbor mesenchymal-like stem/progenitor cells and some of the tissues have been treated as biomedical wastes, for example, exfoliated primary teeth and extracted third molars. We asked whether stem/progenitor cells from discarded dental tissues can be reprogrammed into iPS cells. The 4 factors *Lin28/Nanog/Oct4/Sox2* or *c-Myc/Klf4/Oct4/Sox2* carried by viral vectors were used to reprogram 3 different dental stem/progenitor cells: stem cells from exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), and dental pulp stem cells (DPSCs). We showed that all 3 can be reprogrammed into iPS cells and appeared to be at a higher rate than fibroblasts. They exhibited a morphology indistinguishable from human embryonic stem (hES) cells in cultures and expressed hES cell markers SSEA-4, TRA-1-60, TRA-1-80, TRA-2-49, Nanog, Oct4, and Sox2. They formed embryoid bodies in vitro and teratomas in vivo containing tissues of all 3 germ layers. We conclude that cells of ectomesenchymal origin serve as an excellent alternative source for generating iPS cells.

Introduction

The foundation of cell-based therapy lies in the technologies of procuring cells, especially stem cells. Pluripotent embryonic stem (ES) cells are the most promising cell source for cell-based therapy in regenerative medicine as they give rise to cells of all germ layers and their supply is potentially unlimited. Recent development of generating induced pluripotent stem (iPS) cells by introducing 4 factors: *c-Myc, Klf4, Oct4, and Sox2* [1–2] or *Lin28, Nanog, Oct 4, and Sox2* [3] into somatic cells has shed light on the possibility of obtaining autologous pluripotent embryonic-like stem cells circumventing the need of dealing with nuclear transfer and embryos [1–3]. The initial establishment of human iPS cells was based on the reprogramming of dermal fibroblasts (DFs) with the understanding that dermal tissue is easy to access. Other types of cells in the mouse system such as subpopulation of neural stem cells have been found to be easily reprogrammed with <4 factors [4–6]. However, from the perspective of clinical applications, neural stem cells are not easily accessible if autologous human iPS cells are to be generated. Because the introduction of these factors has been via viral vectors, significant efforts have been put into removing the vectors from cells after they are being reprogrammed into iPS cells [7–11]. Nonetheless, any approach that involves the use of vector systems, even after they are removed, poses some uncertainty on their safety.

To completely circumvent the use of vectors, delivery of recombinant protein-based 4 factors to generate iPS cells in the

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Feeder-Free Derivation of Induced Pluripotent Stem Cells From Human Immature Dental Pulp Stem Cells

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Induced pluripotent stem cells (iPSCs) can be created by forcing expression of certain genes in fibroblasts or other somatic cell types, reversing them to a pluripotent state similar to that of embryonic stem cells (ESC). Here, we used human immature dental pulp stem cells (hIDPSCs) as an alternative source for creating iPSC. hIDPSCs can be easily isolated from accessible tissue of young and adult patients. hIDPSCs possess a fibroblast-like morphology, retaining characteristics of adult multipotent stem cells. Reprogramming of hIDPSCs was fast, producing primary hIDPSC-iPSC colonies even under feeder-free conditions. hIDPSCs acquired ESC-like morphology, expressed pluripotent markers, possessed stable, normal karyotypes, and demonstrated the ability to differentiated in vitro and in vivo. Our data demonstrate that hIDPSCs-iPSCs offer an advantageous cell system for future cell therapy and basic studies, particularly as a model for pediatric developmental disorders.

Key words: Induced pluripotent stem cells (iPSCs); Pediatric diseases; Human immature dental pulp stem cells (hIDPSCs); Reprogramming

INTRODUCTION

Induced pluripotent stem cells (iPSCs) can be derived from several adult tissues (1,10,19,30,31,33,35,37). Research involving production of iPSCs is being developed around the world and will likely stimulate the development of several areas of biology and medicine. After reprogramming, somatic cells acquire properties of embryonic stem cells (ESCs) in respect to morphology, proliferation, gene expression, epigenetic profile, and differentiation potential (32). This approach allows for the creation of patient-specific pluripotent stem cells, advantageous for future cell therapy due to immune compatibility. Human iPSCs have been derived from skin fibroblasts, keratinocytes, blood progenitors, and from several types of adult stem cells, such as adipose, cord blood, neural, and dental pulp (1,10,19,22,30–32, 36,37). Production of iPSCs opens new opportunities for increased understanding of human genetic diseases and embryogenesis, and will likely have a great impact on future drug screening and toxicological tests (21).

However, the reprogramming methodology for derivation of iPSCs is relatively new and needs refining in terms of technique, efficiency, and cell type choice. For example, reprogramming efficiency reported for human fibroblasts is relatively low, while the reprogramming process for keratinocytes generates iPSC colonies 100-fold more efficiently and twofold faster compared to human fibroblasts. Such difference in efficiency is probably because keratinocytes have expression levels of stem cell-related genes more similar to ESCs than fibroblasts (1,12). The timing of reprogramming and the factors re-

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Polycistronic lentivirus induced pluripotent stem cells from skin biopsies after long term storage, blood outgrowth endothelial cells and cells from milk teeth

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ABSTRACT

The generation of human induced pluripotent stem cells (hiPSCs) requires the collection of donor tissue, but clinical circumstances in which the interests of patients have highest priority may compromise the quality and availability of cells that are eventually used for reprogramming. Here we compared (i) skin biopsies stored in standard physiological salt solution for up to two weeks (ii) blood outgrowth endothelial cells (BOECs) isolated from fresh peripheral blood and (iii) children's milk teeth lost during normal replacement for their ability to form somatic cell cultures suitable for reprogramming to hiPSCs. We derived all hiPSC lines using the same reprogramming method (a conditional (FLPe) polycistronic lentivirus) and under similar conditions (same batch of virus, fetal calf serum and feeder cells). Skin fibroblasts could be reprogrammed robustly even after long-term biopsy storage. Generation of hiPSCs from juvenile dental pulp cells gave similar high efficiencies, but that of BOECs was lower. In terms of invasiveness of biopsy sampling, biopsy storage and reprogramming efficiencies skin fibroblasts appeared best for the generation of hiPSCs, but where non-invasive procedures are required (e.g. for children and minors) dental pulp cells from milk teeth represent a valuable alternative.

1. Introduction

Human induced pluripotent stem cells (hiPSCs) generated from patients with genetic diseases hold great promise for disease modeling, safety pharmacology and drug discovery (Dambrot et al., 2011; Davis et al., 2011; Freund and Mummery, 2009). This is particularly relevant for cells of the internal organs, for which biopsies are not routinely available and therefore analysis of the disease phenotype is hampered. hiPSCs are similar to human embryonic stem cells (hESCs) (Yamanaka, 2012) in that they self-renew and can differentiate into all somatic cell types of the human body.

Since the first derivation of hiPSCs in 2007 using fibroblasts cultured from skin biopsies and the retroviral expression of four pluripotency genes *Oct3/4*, *Sox2*, *Klf4* and *c-Myc* (Takahashi et al., 2007), considerable research has been devoted to reprogramming other somatic cell types, also using other methods of gene delivery to the host cell. These include integrating methods (e.g. using lentiviruses or transposons) and a variety of non-integrating approaches (adenovirus, plasmid, protein, episomal vectors and RNA; reviewed in Tiscornia et al. (2011)). The obvious advantages of non-integrating methods are still limited by their relatively low efficiencies, high cost and labor intensity. In addition, general transfection methods require relatively large numbers of somatic cells. Integrating methods by contrast are reasonably efficient but the quality of the resultant iPSC lines may

Abbreviations: hESC, human embryonic stem cells; (h)iPSC, (human) induced pluripotent stem cell; BOECs, blood outgrowth endothelial cells; DMEM, Dulbecco's Modified Eagle Media; PBS, phosphate buffered saline; MEF, mouse embryonic fibroblast; AP, alkaline phosphatase; MOI, multiplicity of infection; NEAA, non-essential amino acids; FCS, fetal calf serum; KOSR, knock-out serum replacement; AFP, α-fetoprotein

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Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts

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Background and Objective: Human induced pluripotent stem (iPS) cells, which have similar properties to human embryonic stem (hES) cells, have been generated from neonatal and adult human dermal fibroblasts by reprogramming. iPS cells have high pluripotency and differentiation potential, and may be a potential autologous stem cell source for future regenerative therapy.

Material and Methods: iPS cell lines from human gingival fibroblasts and, for the first time, from periodontal ligament fibroblasts, were generated by reprogramming using a retroviral transduction cocktail of *OCT3/4, SOX2, KLF4* and c-*MYC*. iPS induction was investigated through expression of the embryonic stem cell markers SSEA4, OCT4, NANOG, GCTM-2, TG30 and TRA-1-60. Following *in vitro* differentiation, the expression of genes for differentiation markers for ectoderm (*SOX1, PAX6*), mesoderm [*RUNX1*, T(*Brachyury*)] and endoderm (*GATA4, AFP*) was assessed by real-time RT-PCR. The ability to form teratomas following implantation into mouse testes was assessed by histology.

Results: Human gingival fibroblast- and periodontal ligament fibroblast-derived iPS cells showed similar characteristics to hES cells. Both sets of iPS cells displayed colony morphology comparable to that of hES cells and expressed the hES cell-associated cell-surface antigens, SSEA3, SSEA4, GCTM-2, TG30 (CD9) and Tra-1-60, and the hES cell marker genes, *OCT4, NANOG* and *GDF3*. These iPS cells showed differentiation potential to form embryoid bodies *in vitro* and expressed genes for endoderm, ectoderm and mesoderm. Teratoma formation following implantation into mouse testes was observed.

Conclusion: These results demonstrate that iPS cells can be successfully generated from adult human gingival and periodontal ligament fibroblasts.

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Key words: periodontal ligament; gingiva; stem cell; induced pluripotent stem cell

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Periodontitis is an infectious inflammatory disease causing the destruction of periodontal tissue and, in serious cases, leads to tooth loss (1). Once the periodontal tissue, including periodontal ligament and alveolar bone, is destroyed it is clinically difficult to reconstruct. As current procedures have shown unpredictable and limited potential for periodontal regeneration, the development of alternative strategies to regenerate

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RESEARCH Open Access

First-in-human study and clinical case reports of the alveolar bone regeneration with the secretome from human mesenchymal stem cells

Wataru Katagiri*, Masashi Osugi, Takamasa Kawai and Hideharu Hibi

Abstract

Background: Secreted growth factors and cytokines in the conditioned medium from bone marrow-derived mesenchymal stem cells (MSC-CM) have several effects on cell behavior. Our previous studies revealed that MSC-CM enhances bone regeneration by increasing cell mobilization, angiogenesis, and osteogenesis in vitro and in vivo. This clinical study was undertaken to evaluate the safety and use of MSC-CM for alveolar bone regeneration in eight patients who were diagnosed as needing bone augmentation prior to dental implant placement.

Methods: The protocol of this clinical study was approved by the ethics committee of Nagoya University Hospital. MSC-CM was prepared from conditioned medium from commercially available human bone marrow-derived MSCs. Patients were treated with beta-tricalcuim phosphate (β-TCP) or an atelocollagen sponge soaked with MSC-CM. Clinical and radiographic assessments were performed during the follow-up period. Histological assessments were also performed in some cases. Clinical and histological data from patients who underwent the SFE procedure without MSC-CM were also used retrospectively as reference controls.

Results: MSC-CM contained several cytokines such as insulin-like growth factor-1, vascular endothelial growth factor, transforming growth factor-β1, and hepatocyte growth factor in relatively low amounts. No systemic or local complications were reported throughout the study. Radiographic evaluation revealed early bone formation in all cases. Histological evaluation also supported the radiographic findings. Furthermore, infiltration of inflammatory cells was scarce throughout the specimens.

Conclusions: MSC-CM was used safely and with less inflammatory signs and appears to have great osteogenic potential for regenerative medicine of bone. This is the first in-human clinical study of alveolar bone regeneration using MSC-CM.

Keywords: Secretome, Mesenchymal stem cells (MSC), Tissue engineering, Regenerative medicine, Bone

Background

Alveolar bone regeneration with grafting is often carried out prior to placement of dental implants. Several graft materials have been used including autogenous bone, xenogeneic bone, and synthetic bone substitutes. Autogenous bone grafts have been used for a long time with good predictability and are considered the "gold standard" because of their osteoinductive and osteoconductive properties and immunogenic compatibility. However, autogenous bone must be harvested from a donor site of the patient and is associated with higher morbidity [1, 2]. Xenogeneic bone and synthetic bone substitutes such as deproteinized bovine bone, hydroxyapatite, and calcium triphosphate are often used clinically as osteoconductive scaffolds, but they provide limited osteoinductivity and a potential risk of infection and extrusion [3]. Osteoinductive growth factors such as bone morphogenic protein (BMP)-2 have been used with these osteoconductive materials to promote bone regeneration [4]. However, recent studies have indicated unexpected effects on bone regeneration [5] including induction of a severe inflammatory response,

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Case Report

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Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: A case report

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Abstract

Objective: To report a case of successful allogeneic grafting of mesenchymal dental pulp stem cells (DPSCs) as preliminary findings in a patient with periodontal disease enrolled into clinical trial ISRCTN12831118.

Methods: Mesenchymal stem cells from the dental pulp of a deciduous tooth from a 7-year-old donor were separated from the pulp chamber and processed via enzymatic digestion and centrifugation. DPSCs were passaged and cultured on a 35 × 13 mm culture dish in minimum essential medium-alpha, without supplementation. After reaching 80% confluency, 5 x 10^6 allogeneic DPSCs in 250 μl phosphate buffered saline were seeded onto a dry scaffold of lyophilized collagen-polyvinylpyrrolidone sponge placed in the left lower premolar area of a 61-year-old patient with periodontal disease. Surgical access to the lower premolar area was achieved using the flap technique.

Results: At 3 and 6 months following allogeneic graft, the patient showed no sign of rejection and exhibited decreases in tooth mobility, periodontal pocket depth and bone defect area. Bone mineral density had increased at the graft site.

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RANDOMIZED CLINICAL TRIAL

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Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial

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Abstract

Aim: The goal of this study was to evaluate if dental pulp stem cells (DPSCs) delivered into intrabony defects in a collagen scaffold would enhance the clinical and radiographic parameters of periodontal regeneration.

Materials and Methods: In this randomized controlled trial, 29 chronic periodontitis patients presenting one deep intrabony defect and requiring extraction of one vital tooth were consecutively enrolled. Defects were randomly assigned to test or control treatments which both consisted of the use of minimally invasive surgical technique. The dental pulp of the extracted tooth was mechanically dissociated to obtain micrografts rich in autologous DPSCs. Test sites ($n = 15$) were filled with micrografts seeded onto collagen sponge, whereas control sites ($n = 14$) with collagen sponge alone. Clinical and radiographic parameters were recorded at baseline, 6 and 12 months postoperatively.

Results: Test sites exhibited significantly more probing depth (PD) reduction (4.9 mm versus 3.4 mm), clinical attachment level (CAL) gain (4.5 versus 2.9 mm) and bone defect fill (3.9 versus 1.6 mm) than controls. Moreover, residual PD < 5 mm (93% versus 50%) and CAL gain ≥4 mm (73% versus 29%) were significantly more frequent in the test group.

Conclusions: Application of DPSCs significantly improved clinical parameters of periodontal regeneration 1 year after treatment.

KEYWORDS

dental pulp, periodontal pocket, periodontal regeneration, randomized controlled trial, stem cells, tissue engineering

1 | INTRODUCTION

The goal of periodontal therapy is to arrest disease progression and ultimately to regenerate lost periodontal tissues (Karring, Nyman, Gottlow, & Laurell, 1993). Several studies over the past 30 years had demonstrated that blood clot stability plays a pivotal role in regeneration of tooth-supporting tissues (Wikesjo & Nilveus, 1990; Wikesjo et al., 2003), avoiding apical migration of epithelial cells during the first healing phase. In this respect, new surgical techniques designed to optimize flap and clot stability (Cortellini & Tonetti, 2007, 2009; Harrel, Nunn, & Belling, 1999; Trombelli, Farina, Franceschetti, & Calura, 2009) and new

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Effects of bone marrow-derived mesenchymal stem cells and platelet-rich plasma on bone regeneration for osseointegration of dental implants: Preliminary study in canine three-wall intrabony defects

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Abstract: Tissue engineering has been applied to overcome the obstacles encountered with bone regeneration for the placement of dental implants. The purpose of this study was to determine the bone formation ability of human bone marrow-derived mesenchymal stem cells (BMMSCs) and platelet-rich plasma (PRP) when applied separately or together to the intrabony defect around dental implants with a porous hydroxyapatite (HA) scaffold. Standardized three-wall intrabony defects (4 × 4 × 4 mm) were created at the mesial of each dental implant site in four mongrel dogs. Defects were then grafted with the following materials: HA + BMMSCs (HS group), HA + PRP (HP group), HA + BMMSCs + PRP (HSP group), and HA scaffold alone (HA group). The level of bone formation (bone density) and osseointegration (bone-to-implant contact [BIC]) in bone defects around the implants were evaluated by histological and histometric analysis at 6 and 12 weeks after the placement of implants. HA, HS, HP, and HSP groups generally showed an increase in bone density and BIC between 6 and 12 weeks, except BIC in the HS group. Although no statistically significant differences were found among HA, HS, HP, and HSP groups ($p > 0.05$), the highest level of bone density and BIC were observed in the HSP group after the 12-week healing period. Furthermore, the level of bone maturation was higher in the HSP group than in the other groups as determined histologically. The findings of this preliminary study suggest that BMMSCs and PRP combined with HA scaffold may provide additional therapeutic effects on bone regeneration and improve osseointegration in bone defects around dental implants. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 102B: 1021–1030, 2014.

Key Words: bone regeneration, dental implant, bone marrow-derived mesenchymal stem cells, platelet-rich plasma, hydroxyapatite

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INTRODUCTION

Dental implantation has become one of the most common methods for restoring defects caused by tooth loss. However, in cases of alveolar bone loss, bone reconstruction is necessary for the successful placement of implants to avoid functional and esthetic problems. Autogenous, allogenic, xenogenic, and synthetic bone have been used as bone grafting materials for the restoration of bone defects. As autogenous bone grafts include living cells, such as osteoblasts and osteoprogenitor cells, and lack immune responses, they are considered more advantageous for forming new bone tissues compared with other bone grafting materials. However, they require additional surgery for harvesting the graft materials and a prolonged procedure time, which may cause inflammation. Moreover, sometimes it is not possible to obtain enough autogenous bone to fill the bone defect area.[1] In cases of allogenic and xenogenic bones, the risk of developing AIDS and contagious diseases via contaminated blood,

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Chapter 14

Generation of a Bioengineered Tooth by Using a Three-Dimensional Cell Manipulation Method (Organ Germ Method)

Masamitsu Oshima, Miho Ogawa, Masato Yasukawa, and Takashi Tsuji

Abstract

The arrangement of cells within a tissue plays an essential role in organogenesis, including tooth development. Organ morphogenesis and physiological functions induced by three-dimensional tissue organization are well known to be regulated by the proper spatiotemporal organization of various signaling molecules, including cytokines, extracellular matrix proteins, and adhesion molecules. Development of a three-dimensional cell manipulation technology to create a bioengineered organ germ, designated as the organ germ method, enabled the generation of a structurally correct and fully functional bioengineered tooth in vivo. This method is expected to be utilized as a valuable technique for analyzing gene and protein functions during organogenesis. Here, we describe protocols for tooth germ reconstitution using the organ germ method and methods for analyzing tooth development in vitro and in vivo.

Key words: Bioengineered tooth, Organ germ method, Cell manipulation, Transplantation, Tooth germ

1. Introduction

The tooth is an ectodermal organ arising from the tooth germ, which is induced by reciprocal interactions between the oral epithelium and mesenchyme in the developing embryo (1–3). Following tooth germ formation, the epithelial and mesenchymal cells in the tooth germ differentiate into the cells, which form tooth tissue, including ameloblast, odontoblast, and pulp cells, as well as periodontal ligament cells (4, 5). These cells secrete the minerals that create hard tissues, such as enamel, dentin, cementum, and alveolar bone (4, 5). Various morphological features

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Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine

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Mesenchymal stem cell-mediated tissue regeneration is a promising approach for regenerative medicine for a wide range of applications. Here we report a new population of stem cells isolated from the root apical papilla of human teeth (SCAP, stem cells from apical papilla). Using a minipig model, we transplanted both human SCAP and periodontal ligament stem cells (PDLSCs) to generate a root/periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function. This work integrates a stem cell-mediated tissue regeneration strategy, engineered materials for structure, and current dental crown technologies. This hybridized tissue engineering approach led to recovery of tooth strength and appearance.

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INTRODUCTION

Regeneration of a functional and living tooth is one of the most promising therapeutic strategies for the replacement of a diseased or damaged tooth [1–3]. Recent advances in dental stem cell biotechnology and cell-mediated murine tooth regeneration have encouraged researchers to explore the potential for regenerating living teeth with appropriate functional properties [4–6]. Murine teeth can be regenerated using many different stem cells to collaboratively form dental structures *in vivo* [4,5,7]. In addition, dentin/pulp tissue and cementum/periodontal complex have been regenerated by human dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs), respectively, when transplanted into immunocompromised mice [8,9]. However, owing to the complexity of human tooth growth and development, the regeneration of a whole tooth structure including enamel, dentin/pulp complex, and periodontal tissues as a functional entity in humans not possible given available regenerative biotechnologies.

The spatially and temporally organized microenvironment of the tooth bud and its surrounding tissues permits growth and development of the crown and roots, resulting in formation and eruption of the tooth [10]. Root development involves dentin formation, cementum generation, instruction of epithelium, and tooth eruption. From a clinical perspective, the most important part of the tooth is the root which supports for a (natural or artificial) crown. The crown alone cannot fulfill normal tooth function without a viable root. In contrast, the wide use of synthetic crowns to replace a damaged natural crowns has been widely applied in dental clinics with excellent therapeutic outcomes [11].

Although dental implant therapies have achieved long-term success in the clinic for the recovery of tooth function, the dental implants require pre-existing high-quality bone structures for supporting the implants [12,13]. Reconstruction of teeth in patients without adequate bone support would be a major advance. Stem cell-mediated root regeneration offers opportunities to regenerate a bio-root and its associated periodontal tissues, which are necessary for maintaining the physiological function of teeth. The purpose of this study is to explore the potential for reconstructing a functional tooth in miniature pigs (minipigs), in which a bio-root periodontal complex is built up by postnatal stem cells including stem cells from root apical papilla (SCAP) and PDLSCs, to which an artificial porcelain crown is affixed. This hybrid strategy of autologous dental stem cell engineering may be applicable to human tooth regeneration. Furthermore, functional tooth restoration in swine may shed light on human tooth regeneration in the future because of the close similarities between swine and human dental tissues [14,15].

RESULTS

Isolation and transplantation of SCAP

The mechanism of the contribution of stem progenitors to root formation remains to be elucidated. Here, we found that human root apical papilla tissue on the exterior of the root foramen area demonstrated positive staining for mesenchymal stem cell surface

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Competing Interests: The authors have declared that no competing interests exist.

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Functional Tooth Restoration by Allogeneic Mesenchymal Stem Cell-Based Bio-Root Regeneration in Swine

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Our previous proof-of-concept study showed the feasibility of regenerating the dental stem cell-based bioengineered tooth root (bio-root) structure in a large animal model. Here, we used allogeneic dental mesenchymal stem cells to regenerate bio-root, and then installed a crown on the bio-root to restore tooth function. A root shape hydroxyapatite tricalcium phosphate scaffold containing dental pulp stem cells was covered by a Vc-induced periodontal ligament stem cell sheet and implanted into a newly generated jaw bone implant socket. Six months after implantation, a prefabricated porcelain crown was cemented to the implant and subjected to tooth function. Clinical, radiological, histological, ultrastructural, systemic immunological evaluations and mechanical properties were analyzed for dynamic changes in the bio-root structure. The regenerated bio-root exhibited characteristics of a normal tooth after 6 months of use, including dentinal tubule-like and functional periodontal ligament-like structures. No immunological response to the bio-roots was observed. We developed a standard stem cell procedure for bio-root regeneration to restore adult tooth function. This study is the first to successfully regenerate a functional bio-root structure for artificial crown restoration by using allogeneic dental stem cells and Vc-induced cell sheet, and assess the recipient immune response in a preclinical model.

Introduction

Tooth loss due to periodontal disease, dental caries, trauma, or a variety of genetic disorders continues to adversely affect most adults in their lives. Regenerative medicine and tissue engineering technologies offer promising therapies for medicine and dentistry [1,2]. Recent advances in dental stem cell biotechnology and cell-based murine tooth regeneration have encouraged researchers to explore the potential for regenerating living functional teeth [3]. However, owing to the complexity of human tooth growth and development, much more researches were needed to regenerate a whole tooth structure, including enamel, dentin/pulp complex, and periodontal tissues as a functional entity. A tooth root that can support a natural or artificial crown is the most important part of the tooth in maintaining tooth functions [4]. Previously, we showed the potential that autologous dental stem cells may be able to form a bioengineered tooth root (bio-root) for temporally supporting artificial crowns in a miniature pig (minipig) as proof of concept preliminary data [5,6]. However, most patients are aged, and sources of autologous dental stem cells are limited. Due to the low immunogenicity and immunomodulation function, allogeneic mesenchymal stem cells (MSCs) could treat systemic lupus erythematosus mice/human [7], Sjögren's syndrome [8], and periodontitis-induced bone defects [9], suggesting that allogeneic MSCs have the potential for dental tissue regeneration. In the present study, we regenerated a functional bio-root using allogeneic dental MSCs and Vc-induced cell sheet, developed a standard stem cell procedure for bio-root regeneration and function tooth restoration in a swine model.

Design and Methods

Animals

This study was reviewed and approved by the Animal Care and Use Committees of the Capital Medical University. Eighteen inbred miniature pigs (minipigs) aged 18 months and weighing 50–60 kg were obtained from the Institute of Animal Science at the Chinese Agriculture University. Animals were housed under conventional conditions with free access to water and food. These animals were randomly divided into three groups: (1) the hydroxyapatite tricalcium

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Journal of Clinical Periodontology

Tissue-engineered ligament: implant constructs for tooth replacement

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Gault P, Black A, Romette J-L, Fuente F, Schroeder K, Thillou F, Brune T, Berdal A, Wurtz T. Tissue-engineered ligament: implant constructs for tooth replacement. J Clin Periodontol 2010; 37: 750–758. doi: 10.1111/j.1600-051X.2010.01588.x.

Abstract

Aim: A tissue-engineered periodontal ligament (PDL) around implants would represent an important new therapeutic tool to replace lost teeth. The PDL is the key to tooth anchoring; it connects tooth root and alveolar bone, and it sustains bone formation.

Materials and Methods: Cells were isolated from PDL and cultured in a bioreactor on titanium pins. After the formation of multiple cellular layers, pins were implanted in enlarged dental alveolae.

Main Outcome Measures: Cell-covered implants integrated without adverse effects, and induced bone in their vicinity.

Results: A histological examination of a dog model revealed that cells were arranged in a typical ligament-like fashion. In human patients, product safety was ascertained for 6–60 months. Probing and motility assessments suggested that the implants were well integrated with mechanical properties similar to those of teeth. Radiographs demonstrated the regeneration of deficient alveolar bone, the development of a lamina dura adjacent to a mineral-devoid space around the implant and implant migration in an intact bone structure.

Conclusions: New tissue consistent with PDL developed on the surface of dental implants after implantation. This proof-of-principal investigation demonstrates the application of ligament-anchored implants, which have potential advantages over osseointegrated oral implants.

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Key words: alveolar bone; periodontal ligament; tissue engineering; tooth implants

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Apart from its role in tooth anchoring, the periodontal ligament (PDL) provides progenitor cells for alveolar bone formation and remodelling (Devlin & Sloan 2002, Mizuno et al. 2006, Gay et al. 2007); at the bone side facing the tooth root, the PDL plays the role of the periosteum. Periodontal disease with tissue destruction by inflammation often leads to resorption and loss of alveolar bone (Wikesjö et al. 2004, Pinkerton et al. 2008), which may be followed by tooth loss (Cochran 2008). In contrast, a functional PDL induces bone, even at ectopic sites (Hamamoto et al. 2002). The PDL suppresses its own ossification, a biological function associated with the local expression of the homeobox gene *Msx2* (Yoshizawa et al. 2004).

Large, acute PDL and alveolar bone lesions may be repaired when they are induced experimentally; however, care must be taken to protect the repair site from invasion by competing tissues, especially gingival connective tissue (Polimeni et al. 2006). Likewise, tissue repair in periodontal disease is only possible by the protection of the repair site.

A possible approach to the replacement of lost teeth is tissue engineering of the PDL. In support of the feasibility of this concept, the PDL has been shown to possess a capacity for spontaneous regeneration, during which the biomechanical tissue strength is restored (Shionhara et al. 2004), and innervation is re-established (Yamada et al. 1999,

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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Non-Viral Methods For Generating Integration-Free, Induced Pluripotent Stem Cells

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Abstract: Induced pluripotent stem (iPS) cells were created from mouse fibroblasts by induced expression of Yamanaka factors, Oct3/4, Sox2, Klf4, and c-Myc. This technique has quickly resulted in an exponential increase in the amount of pluripotency studies, and has provided a valuable tool in regenerative medicine. At the same time, many methodologies to generate iPS cells have been reported, and are comprised mainly of viral methods and non-viral methods. Although viral methods may not be applicable for clinical applications, various non-viral methods have been reported in recent years, including DNA vector-based approaches, transfection of mRNA, transduction of reprogramming proteins, and use of small molecule compounds. This review summarizes and evaluates these non-viral methods.

Keywords: DNA integration-free, induced pluripotent stem cells, non-viral methods.

INTRODUCTION

Induced pluripotent stem (iPS) cells, which are similar to embryonic stem (ES) cells in morphology, gene expression, epigenetic status, and *in vitro* differentiation, are a type of pluripotent stem cell directly generated from somatic cells by various synthetic methods [1]. Compared with ES cells, iPS cells possess indistinguishable pluripotent capabilities, and their specificity towards patients can bypass some of the risks of ES cells. They are therefore a potential alternative to ES cells in regenerative medicine. Also, they can circumvent ethical concerns. Because iPS cells were originally derived from mouse fibroblasts by retrovirus-mediated introduction of four factors, Oct3/4, Sox2, Klf4, and c-Myc [2], and then reprogrammed from human fibroblasts by the same four factors [3] or by Oct3/4, Sox2, Nanog, Lin28 [4], numerous methods for the generation of these cells have been developed.

Based upon different ways of transforming exogenous genes, the methodology for iPS cell generation can be divided into viral-based methods and non-viral methods. Both these methods may or may not involve integration of exogenous genes into the host genome. Because viral methods may result in gene reactivation and unusual phenotypic expression of iPS cells [5, 6], which could be valuable for further studies and clinical applications, studies using non-viral methods, especially without integration, have been frequently used.

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The following review presents a summary of methods for identification of iPS cells, discusses the current iPS cell generation strategies using non-viral delivery systems which result in DNA free of integration, and describes various applications of this methodology.

METHODS FOR IDENTIFICATION OF IPS CELLS

Compared with differentiated cells, iPS cells contain very different epigenetic signatures. With permissive chromatin, lower levels of heterochromatin, and the frequent appearance of bivalent domains, pluripotent cells are able to differentiate into various tissue types [7]. Currently, three different methods are used for identification.

First, preliminary identification of iPS cells can be based on morphology. Similar to early stage embryonic cells, the chief distinguishing features of iPS cells are small size, high nuclear/cytoplasm ratios, and one or more nuclei. Based upon microstructure, histochemistry, Forssman antigen, and protein synthesis, it has been reported that iPS cells are comprised of more euchromatin, unbound ribosome, and mitochondria, with less organelles and less complexities of cellular structures [8].

Second, immunocytochemistry staining and reverse transcription-polymerase chain reaction (RT-PCR) analysis are essential for identification of iPS cells. Immunological markers of iPS cells include alkaline phosphatase (AKP), stage-specific embryonic antigens (SSEA), Tra-1-60, Tra-1-81 and other molecular labeling techniques [9]. A number of studies have reported that expression of AKP was highly correlated with undifferentiated iPS cells, while negative expression was found in differentiated ES cells [10]. SSEA are glycoproteins expressed in early stage development,

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Review

An Overview of Direct Somatic Reprogramming: The Ins and Outs of iPSCs

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Abstract: Stem cells are classified into embryonic stem cells and adult stem cells. An evolving alternative to conventional stem cell therapies is induced pluripotent stem cells (iPSCs), which have a multi-lineage potential comparable to conventionally acquired embryonic stem cells with the additional benefits of being less immunoreactive and avoiding many of the ethical concerns raised with the use of embryonic material. The ability to generate iPSCs from somatic cells provides tremendous promise for regenerative medicine. The breakthrough of iPSCs has raised the possibility that patient-specific iPSCs can provide autologous cells for cell therapy without the concern for immune rejection. iPSCs are also relevant tools for modeling human diseases and drugs screening. However, there are still several hurdles to overcome before iPSCs can be used for translational purposes. Here, we review the recent advances in somatic reprogramming and the challenges that must be overcome to move this strategy closer to clinical application.

Keywords: embryonic stem cells; adult stem; somatic reprogramming; induced pluripotent stem cells (iPSCs)

1. Introduction

Stem cells are a subset of cells in our body with the remarkable ability to self-renew and differentiate along different cell-lineages [1]. They can be classified into two main categories based on their self-renewing capacity and plasticity, namely "embryonic stem cells" and "non-embryonic" adult/somatic stem cells. Self-renewal refers to the ability to undergo multiple divisions while maintaining an undifferentiated state, while plasticity refers to the ability of a cell to differentiate down multiple different cell lineages. Plasticity can also be referred to as the potency of a cell.

Embryonic Stem Cells (ESCs) have the unique potential to endlessly divide while maintaining an undifferentiated state (self-renewing) but also the capacity to differentiate into all germ layers as well as extra-embryonic tissues or placental cells, being termed as totipotent. Days after fertilization, these totipotent cells mature and form more specialized cells called pluripotent cells (Figure 1). Pluripotent stem cells maintain the ability to self-renew and differentiate into all three germ layers and down many lineages. These cells, through complex mechanisms are responsible for tissue growth, repair and maintenance. The so-called mouse ESCs (mESCs) are isolated at day E3.5 from the inner cell mass of