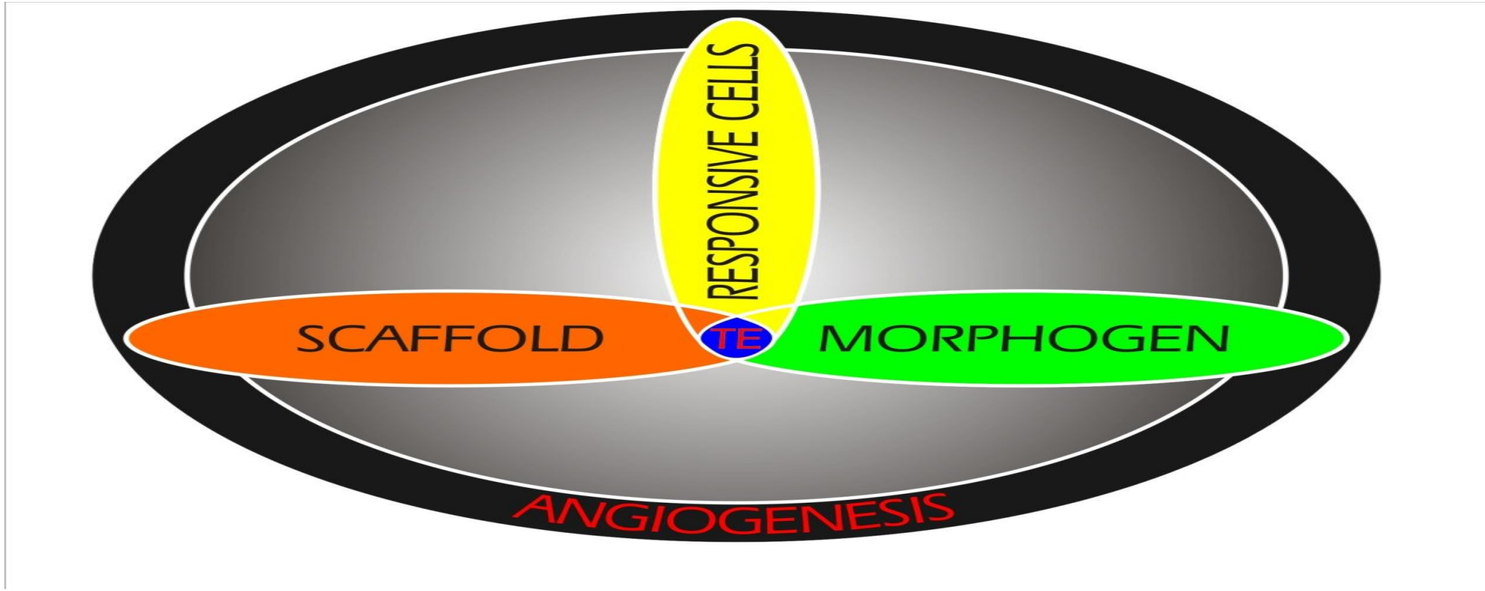
ENGENHARIA TECIDUAL

Prof. Flávio F. Demarco

ENGENHARIA TECIDUAL



Fatores morfogênicos (fatores de crescimento)

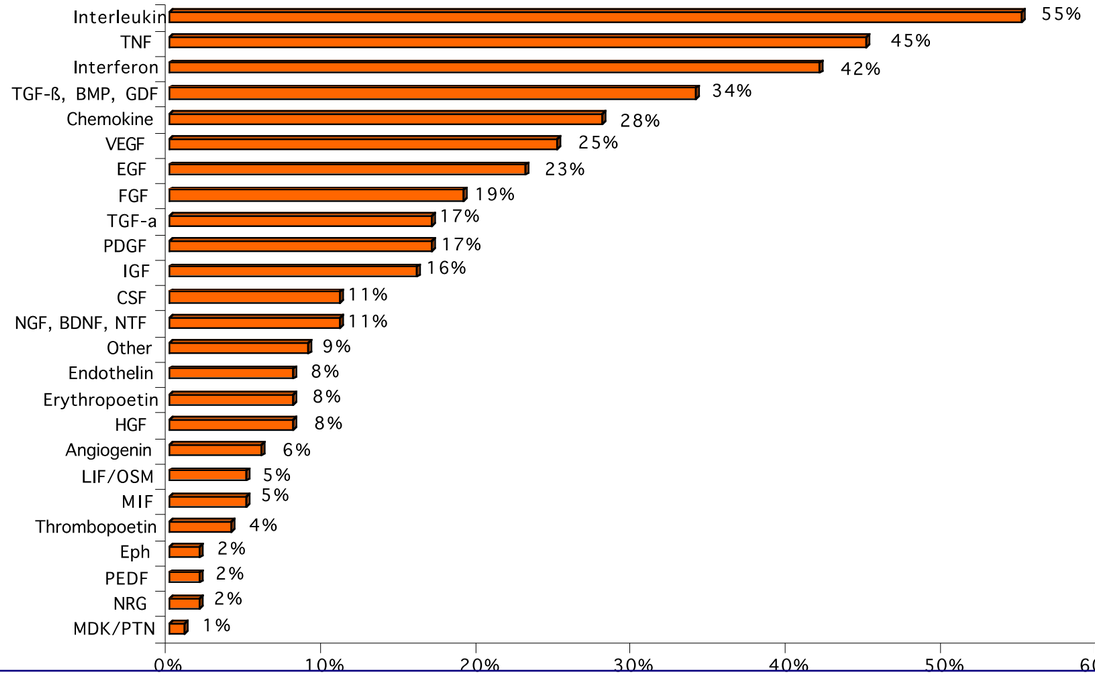
Definição

- Fatores de crescimento são proteínas solúveis que atuam como agentes de sinalização para as células, influenciando funções críticas, tais como: divisão celular, síntese de matriz e diferenciação tecidual, através da união receptor-ligante (Hugs et al., 2006)

Citocinas e Fatores de crescimento

- Diversos papéis no desenvolvimento e na doença
- Estudos incluem receptores e ligantes
- Várias famílias de moléculas e membros da mesma família

Moléculas mais frequentemente estudadas



Fatores de crescimento

- Transforming growth factor
- Bone morphogenetic proteins
- Fibroblast growth factors
- Platelet-derived growth factors
- Insulin-like growth factors

Cell signaling

"Information metabolism"

How cells receive, process and respond to information from the environment

Membran/nuclear receptors

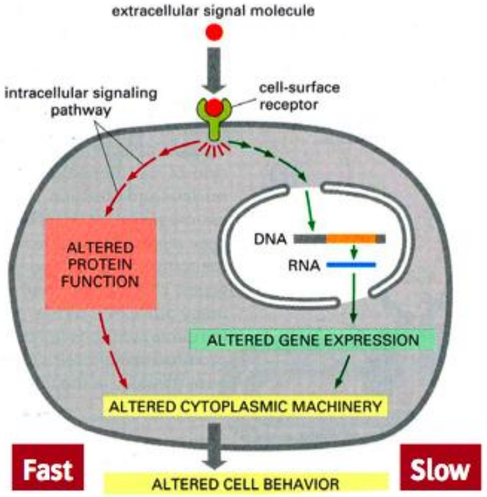
Transfer information from the environment to the cell's interior

Second messengers

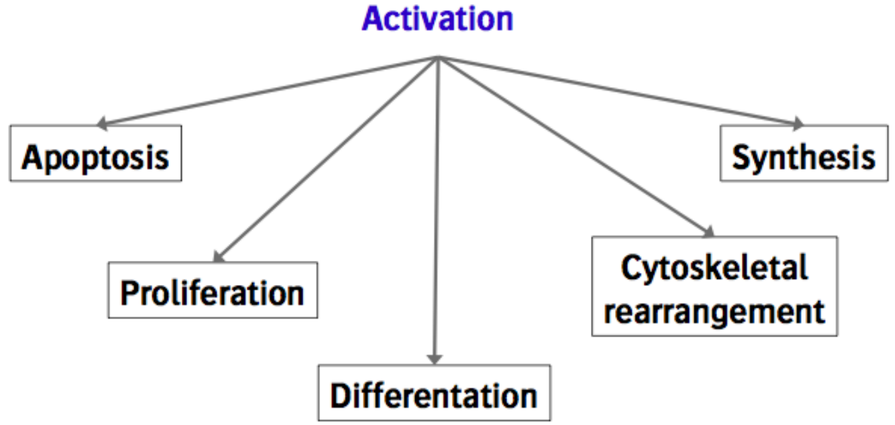
Protein phosphorylation

Activation of protein kinases

Termination of signal



Intracellular signaling pathways



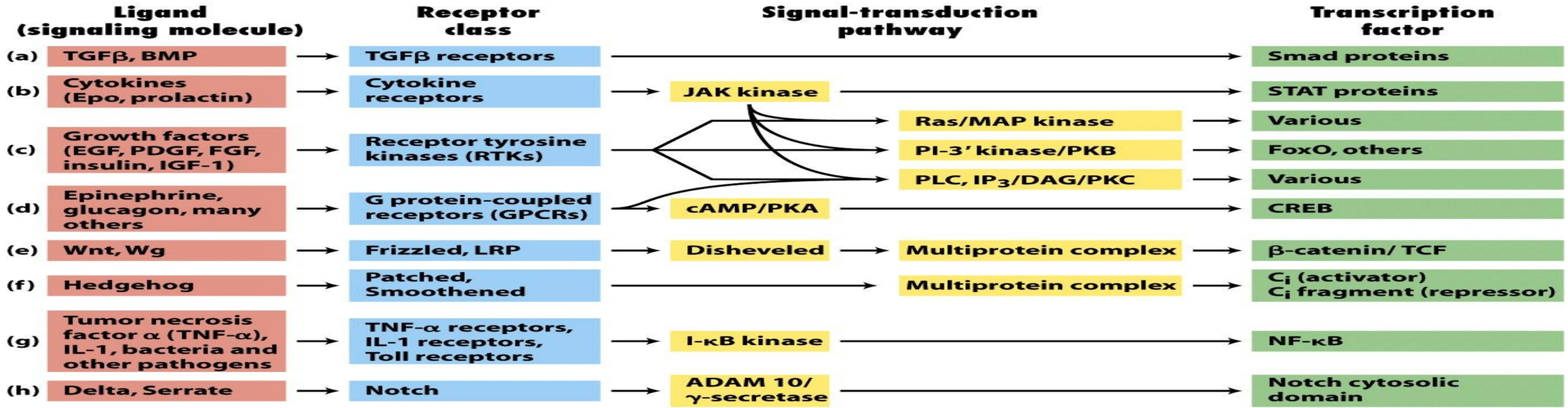


Figure 16-2
Molecular Cell Biology, Sixth Edition

Vitality of the Dentin-Pulp Complex in Health and Disease: Growth Factors as Key Mediators

Anthony J. Smith, Ph.D.

Fatores de crescimento são peptídeos que transmitem sinais entre as células funcionando como estimuladores e/ou inibidores de crescimento, assim como moduladores de diferenciação, dentre outras funções. Tem um papel central em controlar comportamento e a atividade celular.

Podem ter um grau de especificidade em termos das células que eles atuarão, mas alguns são mais versáteis e podem atuar em um grande número de tipos celulares.

A dose-dependência de seus efeitos varia, mas uma de suas características principais é a sua potencial em muito baixa concentração (picogramas).

Journal of Dental Education ■ Volume 67, Number 6

Growth Factors as Cell-Signalling Molecules

Therapeutic Approaches to Dental Tissue Repair and Natural Regeneration as a Blueprint for Tissue Engineering

Development of tissue engineering strategies that exploit the cell-signalling properties of growth factors have the potential for profoundly changing the clinical management of dental disease and the restoration of the dentin-pulp complex.

Journal of Dental Education ■ Volume 67, Number 6

Vitality of the Dentin-Pulp Complex in Health and Disease: Growth Factors as Key Mediators

Anthony J. Smith, Ph.D.

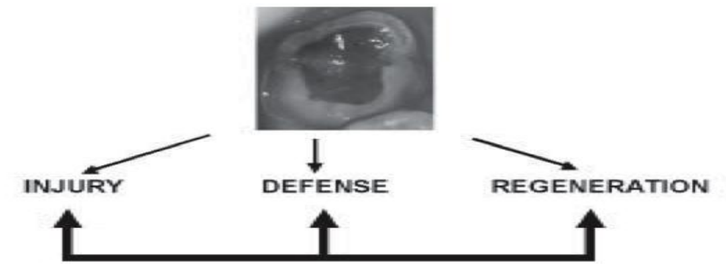


Figure 1. The overall response of the tooth to injury, such as dental caries, represents the complex interplay among injury, defense, and regeneration.

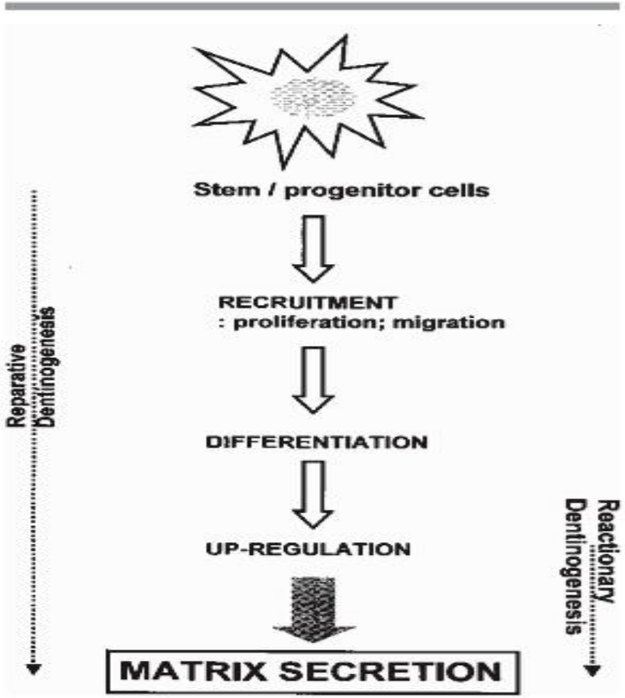


Figure 2. The cellular events involved in reparative and reactionary dentinogenesis

Journal of Dental Education ■ Volume 67, Number 6

Vitality of the Dentin-Pulp Complex in Health and Disease: Growth Factors as Key Mediators

Anthony J. Smith, Ph.D.

Table 1. Superfamilies and families of the more commonly recognized growth factors

Superfamily	Family	Abbreviated name
Transforming growth factor β	Transforming growth factor βs	TGF-β
	Inhibins	Inhibin/Activin
	Bone morphogenetic proteins	BMP
	Vg-1	Vg-1, GDF-1, DPP
Platelet-derived growth factor	Platelet-derived growth factors	PDGF
	Vascular endothelial growth factors	VEGF
	Connective tissue growth factors	CTGF
Epidermal growth factor	Epidermal growth factor	EGF
	Transforming growth factor α	TGF α
Other large peptide growth factor families	Fibroblast growth factors	FGF
	Insulin-like growth factors	IGF
	Nerve growth factor	NGF
	Tumor necrosis factors*	TNF

*TNF-α and TNF-β are usually classified as pro-inflammatory cytokines, but sometimes considered within growth factor classifications.

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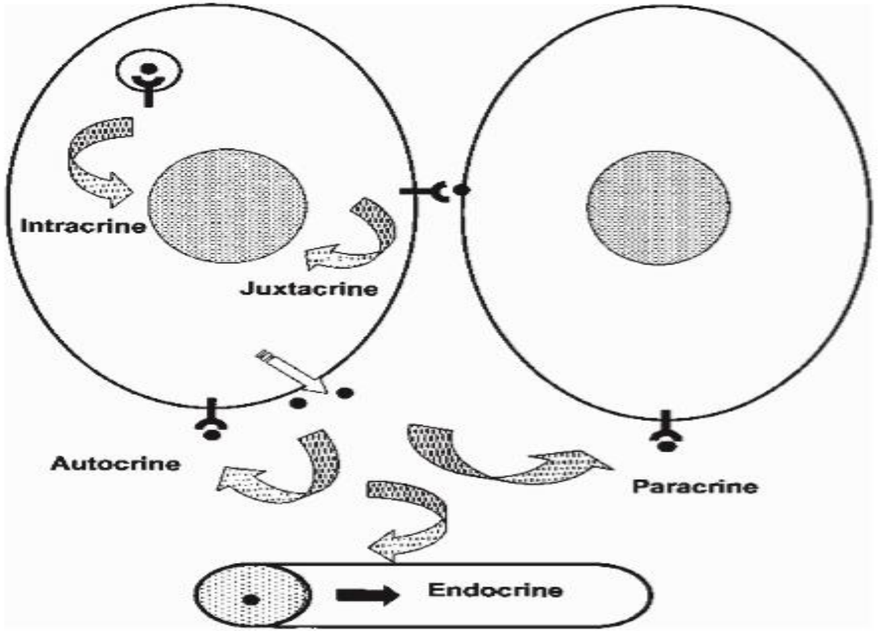

Figure 3. Growth factors may act in endocrine, autocrine, paracrine, juxtacrine, and intracrine modes. Growth factors (black circles) are produced and released from cells and then interact with (via specific receptors) either the cells producing them (autocrine, intracrine) or other cells (paracrine, juxtacrine, endocrine) resulting in the signalling of a response by these cells.

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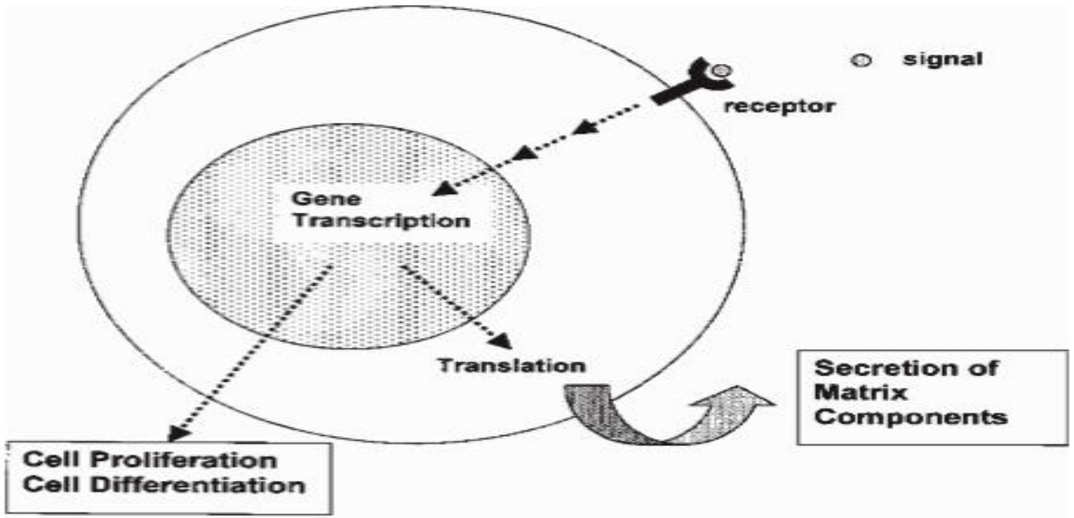

Figure 4. Growth factor interaction with a specific receptor on the cell surface results in signal transduction to the nucleus influencing gene transcription, which can direct a variety of responses including proliferation, differentiation, and matrix secretion.

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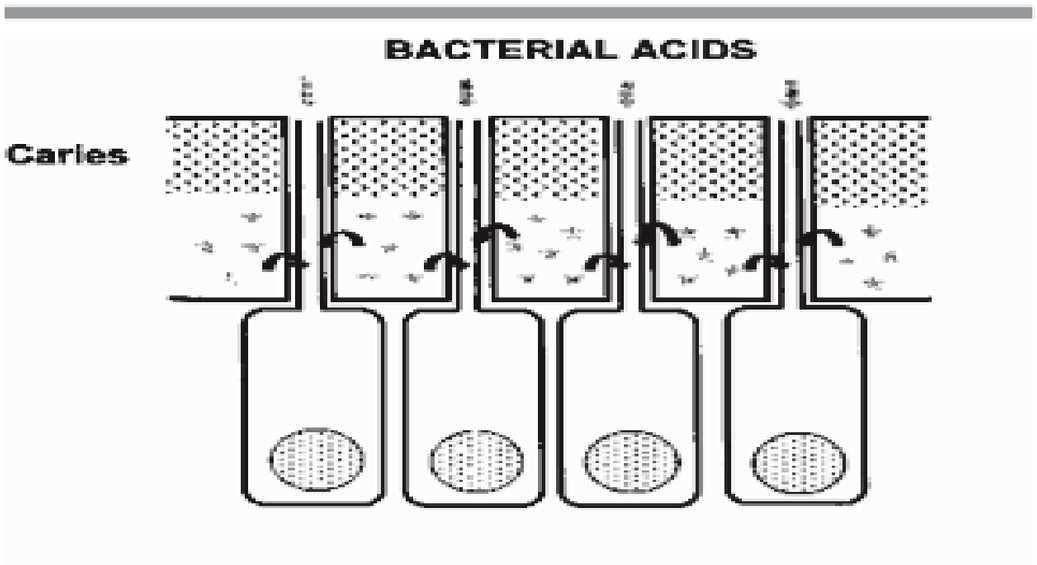

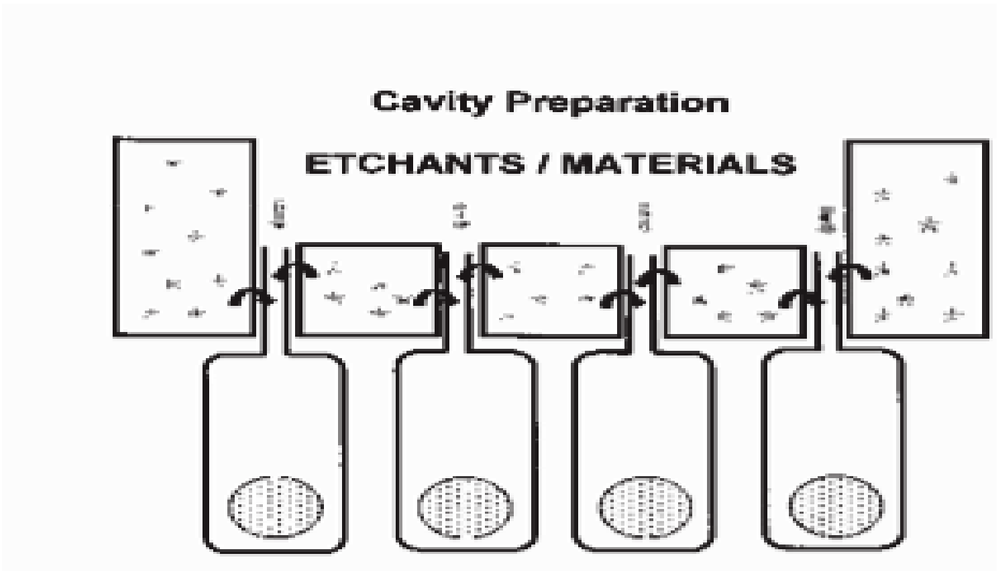

Transforming Growth Factor

- Superfamília de fatores de crescimento (~34 membros)
- Atua nos recepetores de parede celular do tipo cinase serina/trionina (serine/threonine kinase)
- Promove proliferação e diferenciação de células mesenquimais, precursores para osteoblastos, osteoclastos e condrócitos.
- Estimula formação óssea
  - Induz síntese de proteoglicanos específicos de cartilagem e colágeno tipo II
  - Estimula a síntese de colágeno por osteoblastos

TGFβ signalling: a complex web in cancer progression

Hiroaki Ikushima and Kohei Miyazono

NATURE REVIEWS | CANCER VOLUME 10 | JUNE 2010 | 415

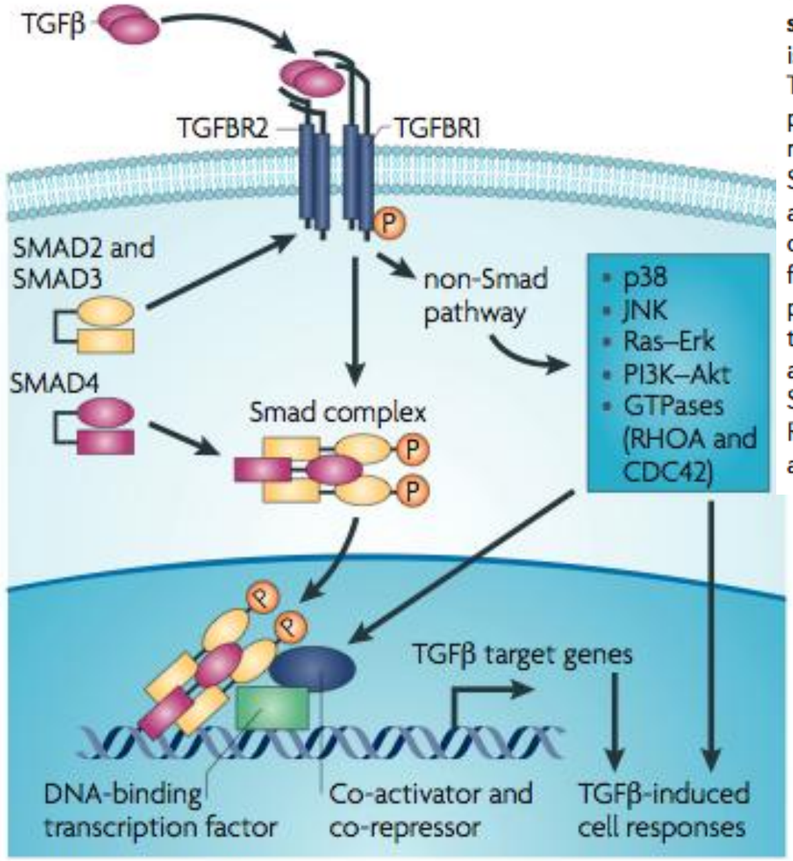

Figure 1 | **Intracellular signal transduction of TGFβ signalling.** Transforming growth factor-β (TGFβ) signalling is transduced through Smad and non-Smad pathways. TGFβ ligand binds to TGFBR2 and TGFBR1. TGFBR2 phosphorylates (P) TGFBR1, which subsequently phosphorylates and activates SMAD2 and SMAD3. Activated SMAD2 and SMAD3 form a Smad complex with SMAD4 and translocate into the nucleus. In the nucleus, the Smad complex interacts with other DNA-binding transcription factors, and co-activators and co-repressors, binds to the promoter regions of TGFβ target genes and regulates the transcription of target genes. TGFβ stimulation also activates other signalling cascades in addition to the Smad pathway. TGFβ receptors activate p38, JNK, Ras–Erk, PI3K–Akt, and small GTPases such as RHOA and CDC42.

Bone Morphogenetic Proteins

-Primeiramente isolada em extratos ácidos de osso humano por URIST in 1965.
Presente em minima quantidade (1mg per kg de osso),necessitanto grande quantidade osso para ser produzida.
- Necessidade de desenvolver BMPs recombinantes humanas- Parte da superfamíla do TGF Beta (43 membros)
- Ao menos 16 diferentes proteínas isoladas
- BMP1 não é parte desta famíla, sendo uma protease pró-colagenosa
- BMPs secretada por osteoblastos induzem a formação de células odontoprogenitoras e estimulam a formação óssea.
- BMPs recombinantes aumentam a quantidade e pureza disponíveis, mas necessitam de 10 x quantidade para dar o mesmo efeito osteogênico que a BMP natural

List of Bone Morphogenetic Proteins

BMP	Known functions	Gene Locus
BMP1	**BMP1 does not belong to the TGF-β family of proteins*. It is a metalloprotease that acts on procollagen I, II, and III. It is involved in cartilage development.	Chromosome: 8; Location: 8p21
BMP2	Acts as a disulfide-linked homodimer and induces bone and cartilage formation. It is a candidate as a retinoid mediator. Plays a key role in osteoblast differentiation.	Chromosome: 20; Location: 20p12
BMP3	Induces bone formation.	Chromosome: 14; Location: 14p22
BMP4	Regulates the formation of teeth, limbs and bone from mesoderm. It also plays a role in fracture repair.	Chromosome: 14; Location: 14q22-q23
BMP5	Performs functions in cartilage development.	Chromosome: 6; Location: 6p12.1
BMP6	Plays a role in joint integrity in adults.	Chromosome: 6; Location: 6p12.1
BMP7	Plays a key role in osteoblast differentiation. It also induces the production of SMAD1. Also key in renal development and repair.	Chromosome: 20; Location: 20q13
BMP8a	Involved in bone and cartilage development.	Chromosome: 1; Location: 1p35-p32
BMP8b	Expressed in the hippocampus.	Chromosome: 1; Location: 1p35-p32
BMP10	May play a role in the trabeculation of the embryonic heart.	Chromosome: 2; Location: 2p14
BMP15	May play a role in oocyte and follicular development.	Chromosome: X; Location: Xp11.2

Growth Factors, December 2004 Vol. 22 (4), pp. 233–241

Taylor & Francis
healthsciences

Bone Morphogenetic Proteins

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[b]*Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78229, USA*

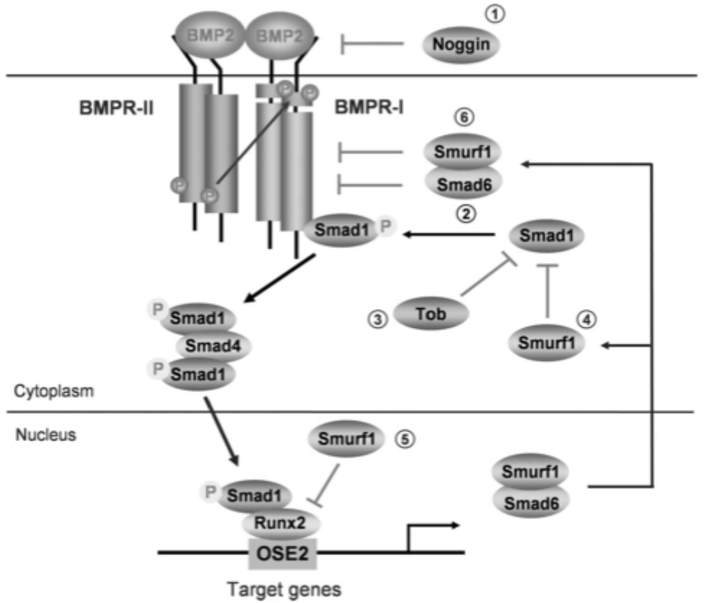


FIGURE 1 BMP signaling and its regulation. BMP signals are mediated by type I and II BMP receptors and their downstream molecules Smad1, 5 and 8. Phosphorylated Smad1, 5 and 8 proteins form a complex with Smad4 and then are translocated into the nucleus where they interact with other transcription factors, such as Runx2 in osteoblasts. BMP signaling is regulated at different molecular levels: (1) Noggin and other cystine knot-containing BMP antagonists bind with BMP-2, 4 and 7 and block BMP signaling. Over-expression of noggin in mature osteoblasts causes osteoporosis in mice (Devlin *et al.*, 2003; Wu *et al.*, 2003). (2) Smad6 binds type I BMP receptor and prevents Smad1, 5 and 8 to be activated (Imamura *et al.*, 1997). Over-expression of Smad6 in chondrocytes causes delays in chondrocyte differentiation and maturation (Horiki *et al.*, 2004). (3) Tob interacts specifically with BMP activated Smad proteins and inhibits BMP signaling. In Tob null mutant mice, BMP signaling is enhanced and bone formation is increased (Yoshida *et al.*, 2000). (4) Smurf1 is a Hect domain E3 ubiquitin ligase. It interacts with Smad1 and 5 and mediates the degradation of these Smad proteins (Zhu *et al.*, 1999). (5) Smurf1 also recognizes bone-specific transcription factor Runx2 and mediates Runx2 degradation (Zhao *et al.*, 2003). (6) Smurf1 also forms a complex with Smad6, is exported from the nucleus and targeted to the type I BMP receptors for their degradation (Murakami *et al.*, 2003). Over-expression of Smurf1 in osteoblasts inhibits postnatal bone formation in mice (Zhao *et al.*, 2004).

Table 2 Temporal and functional characteristics of members of the TGF-β superfamily observed during fracture healing in animal models

Member of the TGF-β superfamily	Time of expression	Specific responses in vivo and in vitro
GDF-8	Restricted to day 1[20]	Potential function as a negative regulator of skeletal muscle growth[20]
BMP-2	Days 1—21[10,20] (the earliest gene to be induced and second elevation during osteogenesis)	Recruitment of mesenchymal cells Chondrogenesis May initiate the fracture healing cascade and regulate the expression of other BMPs BMP-2, -6, -9 may be the most potent to induce osteoblast lineage-specific differentiation of MSCs[19]
BMP-3, -8	Days 14—21[20] (restricted expression during osteogenesis)	Temporal data suggest a role in the regulation of osteogenesis
BMP-4	Transient increased expression in the surrounding soft tissues 6 h to day 5[9] Days 14—21[20] Through out fracture healing[10]	Involvement in the formation of callus at a very early stage in the healing process In vitro: BMP-3 and -4 stimulate the migration of human blood monocytes[63]
BMP-7	Days 14—21[20] From the early stages of fracture healing[9]	Regulatory role in both types of ossification In vitro: stimulation of relative mature osteoblasts[19]
GDF-10, BMP-5, -6	Days 3—21[20]	Regulatory role in both types of ossification BMP-6 may initiate chondrocyte maturation[20]
GDF-5, 1	Day 7 (maximal) to day 14[20] (restricted expression during chondrogenic phase) GDF-1 at extremely low levels	GDF-5 an exclusive involvement in chondrogenesis is suggested Stimulation of mesenchymal aggregation and induction of angiogenesis through chemotaxis of endothelial cells and degradation of matrix proteins
GDF-3, GDF-6, 9	No detectable levels within the fracture callus[20]	GDF-6 may be expressed only in articular cartilage[20] and with GDF-5, 7 more efficiently induce cartilage and tendon-like structures in vivo[28]
TGF-β1, -β2, -β3	Days 1—21[20] Days 3—14[20] Days 3—21[20]	Potent chemotactic for bone forming cells and macrophages Proliferation of undifferentiated mesenchymal and osteoprogenitor cells, osteoblasts, chondrocytes

Table from Dimitriou, et al., Injury, 2005

Antagonistas da BMP

- Podem ter um papel importante na formação óssea
- Noggin
  - Inibidor da Matriz Extra-celular
  - Compete com os receptores de BMP-2

BMPs

Deve ser aplicada localmente devido a rápida degradação sistêmica

Uso através de terapia protéica ou terapia genética

Fibroblast Growth Factors

- Apresenta duas formas: ácida (FGF-1) e básica (FGF-2)
- Aumenta a proliferação de condrócitos e osteoblastos
- Aumenta a formação de calo ósseo
- FGF-2 estimula a angiogênese

Platelet-Derived Growth Factor

- Um dímero, produto de dois genes: PDGF-A and PDGF-B
- Estimula a formação de células ósseas
- Mitogênico para as células de origem mesenquimal
- Aumenta a síntese de colágeno tipo I por aumentar o número de osteoblastos
- PDGF-BB estimula a reabsorção óssea, através do aumento do número de osteoclastos

Insulin-like Growth Factor

- Dois tipos: IGF-I e IGF-II
  - Sintetizado em vários tecidos
  - Producão de IGF-I no fígado é estimulada pelo hormônio do crescimento
- Estimula a síntese de matriz óssea a de produção de colágeno
- Estimula a replicação de osteoblastos
- Inibe a degradação do colágeno

Citocinas

- Interleukin-1,-4,-6,-11, macrophage and granulocyte/macrophage (GM) colony-stimulating factors (CSFs) e Tumor Necrosis Factor
- Estimula a reaborção óssea
  - IL-1 é a mais potente
- Síntese de IL-1 e IL-6 synthesis é diminuída pelo estrogênio
  - Pode ser o mecanismo da reabsorção óssea pós-menopausa
- Pico durante as 1st 24 horas e depois durante a remodelação
- Regula formação óssea endocondral

Estimulação específica para Osteoblastos and Osteoclastos

	Formação óssea	*Reabsorção Óssea*
IL-1	+	+++
TNF-α	+	+++
TNF-β	+	+++
TGF-α	--	+++
TGF-β	++	++
PDGF	++	++
IGF-1	+++	0
IGF-2	+++	0
FGF	+++	0

Prostaglandinas / Leucotrienos

- O efeito na reabsorção óssea é dependente da espécie e seu efeito geral em humanos é desconhecido
- Prostaglandinas da série E
  - Estimula formação de osso osteoblástico
  - Inibe a atividade de osteoclastos isolados
- Leucotrienos
  - Estimula a formação óssea
  - Aumenta a capacidade de alguns osteoclastos para formar pontos de reabsorção

Hormônios

- Estrogênio
  - Estimula a cicatrização de fraturas
  - Modula a liberação do inibitor específico do IL-1
- Hormônio da Paratireóide (PTH)
  - A exposição intermitente estimula osteoblastos e aumenta a formação óssea
- Hormônio do crescimento
  - Mediado através do IGF-1
  - Aumenta a formação do calo e a resistência a fratura

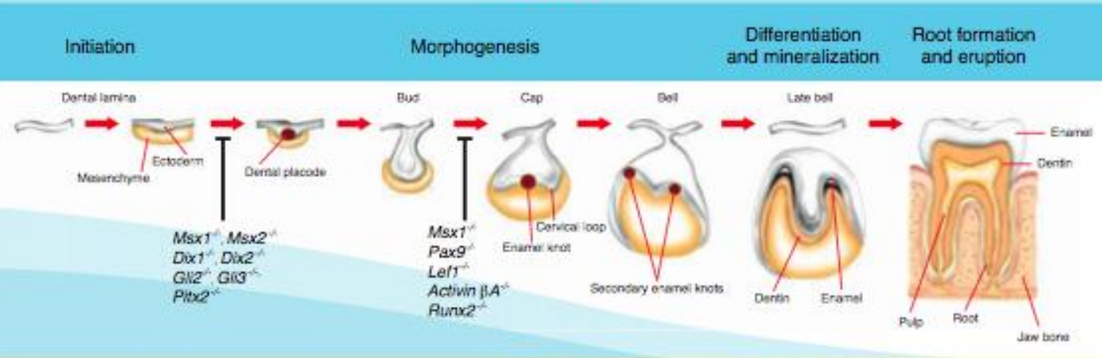
Fatores Vasculares

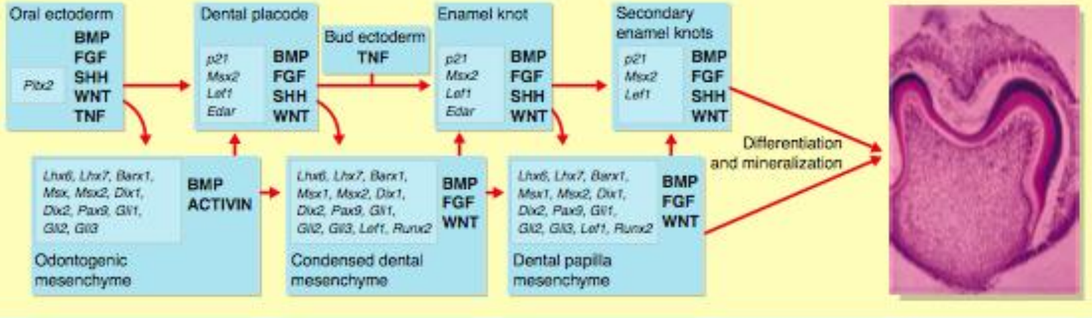
- Metaloproteinases
  - Degradam a cartilagem e o osso para permitir a invasão pelos vasos
- Fatores angiogênicos
  - Vascular-endothelial growth factors (VEGF)
    - Media a neo-angiogênese & mitógenos específicos endoteliais-celulares
  - Angiopoietina (1&2)
    - Regulate formation of larger vessels and branches

Journal of Cell Science

Signalling In Tooth Development

Irma Thesleff





Buonocore Memorial Lecture

Tooth Regeneration ir Operative Dentistry

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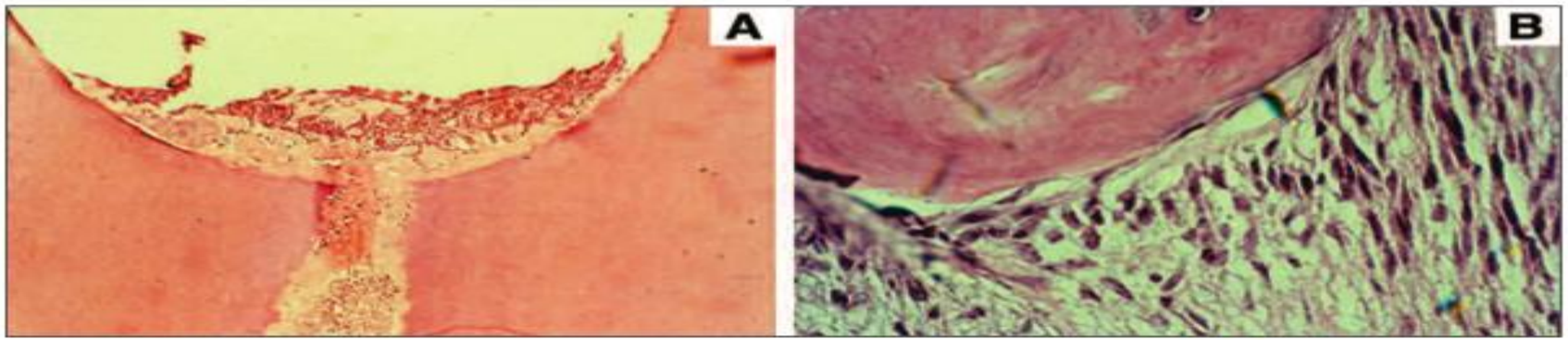

Figure 2. *Reparative dentin induced by BMP-7/OP-1 in monkeys. (A) Reparative dentin induced by OP-1 in the pulp exposure site and in the floor of the cavity preparation. Reparative dentin presents cellular inclusions, 3 weeks after treatment. (B) Reparative dentin induced by BMP-7/OP-1 (high magnification). Reparative dentin is dense and does not present cellular inclusions, 6 months after treatment. Note the absence of signs of inflammatory response in the pulp tissue. These images are courtesy of Dr Bruce Rutherford.*

Buonocore Memorial Lecture

Tooth Regeneration ir Operative Dentistry

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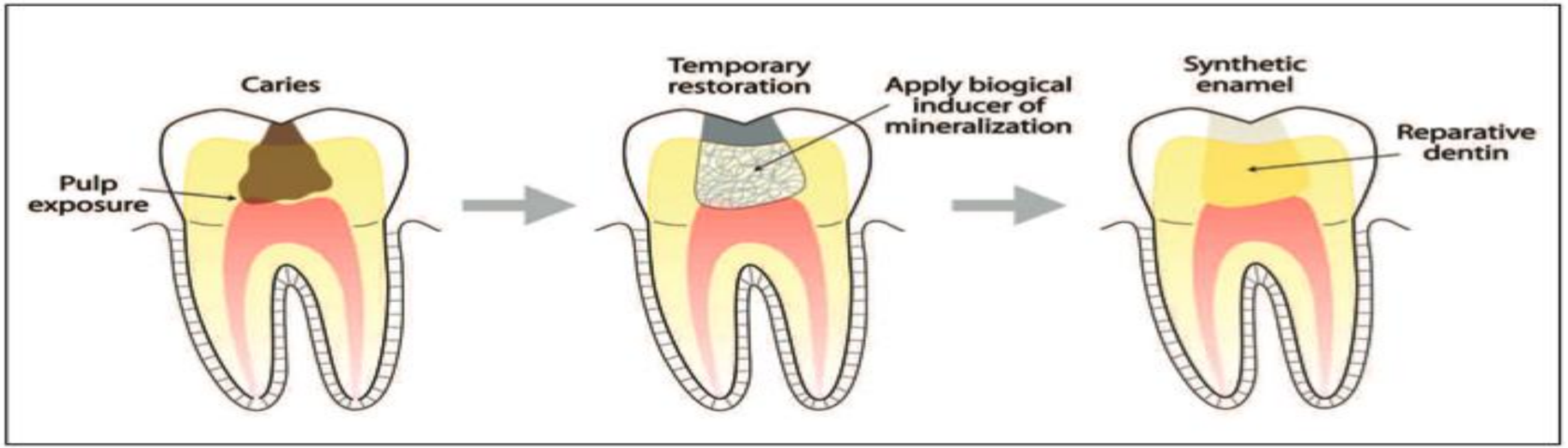

Figure 3. *Diagram depicting the prospect of using a biological inducer of mineralization for dentin regeneration and using synthetic enamel for final restoration of the cavity preparation.*

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Cytokine & Growth Factor Reviews 16 (2005) 369–376
Cytokine Growth Factor Reviews
www.elsevier.com/locate/cytogfr

Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy

Misako Nakashima *

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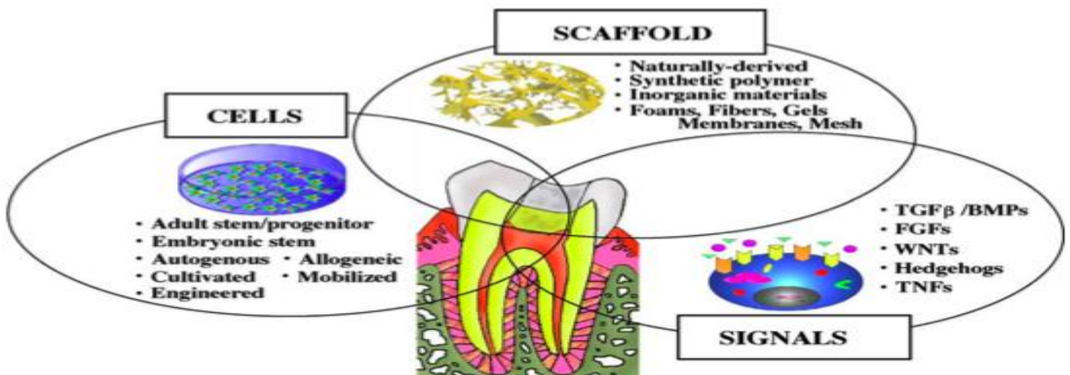

Fig. 1. The key elements of tissue engineering and dentin regeneration. The triad consists of stem cells, a scaffold of extracellular matrix and signals of morphogens.

BMPs
-Usadas sequencialmente e repetidamente durante embriogênese dental, morfogênese, citodiferenciação e secreção da matriz
- Seis diferentes Bmps (Bmp2-Bmp7) são co-expressas temporalmente e espacialmente.

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Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy

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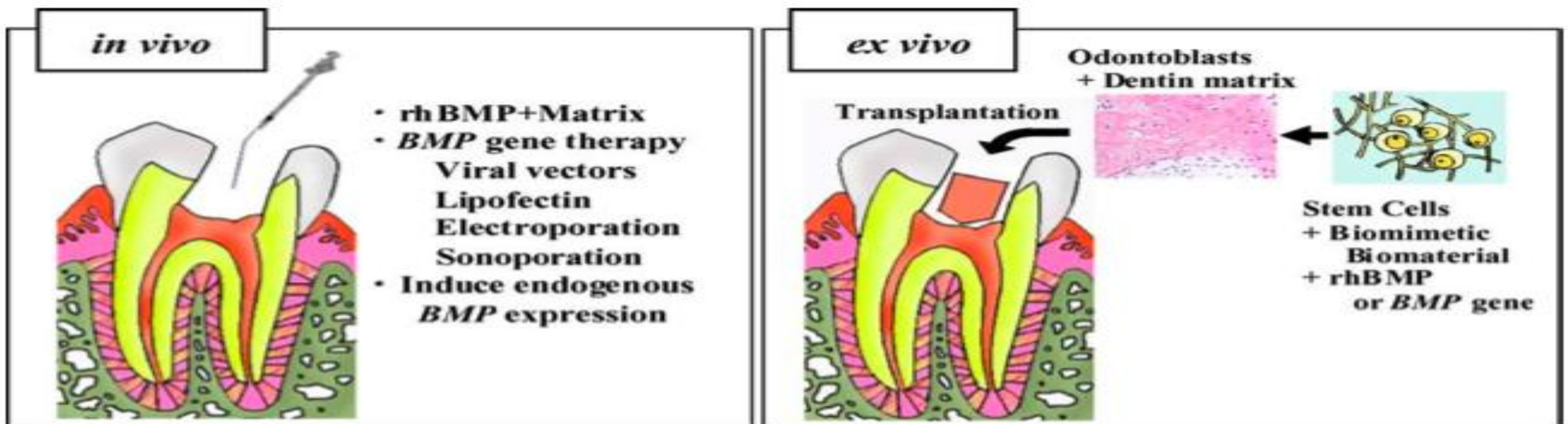

Ultrasom

- Ultasom de baixa intensidade é aprovado pela FDA para estímulo de fraturas recentes
- Modula a transdução de sinal, aumenta e expressão gênica, aumenta o fluxo sanguíneo, aumenta a remodelação óssea e aumenta a resistência torsional do calo em modelos animais

Ultrasom

Arch Oral Biol. 2009 Feb;54(2):185-91. Epub 2008 Nov 5.

VEGF and odontoblast-like cells: stimulation by low frequency ultrasound.

Scheven BA, Man J, Millard JL, Cooper PR, Lea SC, Walmsley AD, Smith AJ.

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Abstract

OBJECTIVE: Vascular endothelial growth factor (VEGF) has been implicated in the regulation of dental pulp and dentine repair. Therapeutic ultrasound was shown to be effective for fracture repair. We investigated whether low frequency ultrasound influences the production of VEGF by odontoblast-like cells. Moreover, we examined the direct effects of VEGF on odontoblast-like cell proliferation. DESIGN: MDPC-23, an established odontoblast-like cell line, was exposed to increasing intensities of 30kHz ultrasound using an ultrasonic tip probe. RESULTS: After 24h cell culture, WST-1 analysis of cell viability and number showed a dose-dependent decrease in the number of viable cells with increasing ultrasound power. However, the relative concentration of VEGF as analysed by ELISA and normalised to cell number was significantly increased in the culture supernatants indicating an ultrasound-induced stimulation of odontoblastic VEGF secretion. Analysis of VEGF gene expression by sqRT-PCR revealed the expression of the main VEGF isoforms in the MDPC-23 cells, i.e. VEGF(120) and VEGF(164) as well as to a minor extent VEGF(188). Low power ultrasound increased gene expression of all VEGF isoforms. Addition of recombinant VEGF to the cell cultures significantly stimulated cell proliferation. Gene expression of the VEGF receptors Flt1/VEGFR1 and KDR/VEGFR2 was detected in the MDPC-23, suggesting the possibility that VEGF may act on the odontoblast-like cells in an autocrine manner. CONCLUSIONS: Our results indicate that ultrasound promoted VEGF expression and production by odontoblast-like cells and that VEGF may have autocrine effects on these cells. It is proposed that ultrasound may influence odontoblast activity and dentine repair by modulating production of endogenous growth factors in the dentine-pulp complex.

Med Hypotheses. 2009 Oct;73(4):591-3. Epub 2009 Jun 23.

Therapeutic ultrasound for dental tissue repair.

Scheven BA, Shelton RM, Cooper PR, Walmsley AD, Smith AJ.

School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, St. Chad's Queensway, Birmingham B4 6NN, UK.
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Abstract

Dental disease affects human health and the quality of life of millions worldwide. Tooth decay (caries) and diseases of the dental pulp result in loss of tooth vitality and function requiring invasive treatment to restore the tooth to health. "Therapeutic" low intensity pulsed ultrasound has been shown to accelerate bone fracture healing indicating that ultrasound may be used as a tool to facilitate hard tissue regeneration. We have shown recently that low frequency ultrasound is able to exert biological effects on odontoblast-like cells. In this paper, we postulate that low frequency, low intensity ultrasound may stimulate endogenous coronal tooth repair by stimulating dentine formation from existing odontoblasts or by activating dental pulp stem cells to differentiate into new reparative dentine-producing cells. Ultrasound therapy promoting dentine formation and repair may also have the potential benefit of alleviating dentine hypersensitivity by inducing occlusion of dentinal tubules. It is envisaged that therapeutic ultrasound may be used in future to facilitate dental tissue engineering and stem cell therapy applications for dental tissue regeneration. Further research is warranted in this clinically important area and we envisage that novel strategies in dental therapy will be realised that may ultimately lead to the development of novel non-invasive, multifunctional ultrasound devices for dental diagnostics, repair and regeneration.

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PERIODONTOLOGY 2000

Regeneration of vascularized bone

Susan X. Hsiong & David J. Mooney

Combined delivery of cells and growth factors

Promoting tissue neovascularization

Endothelial cell transplantation

Delivery of angiogenic factors

Table 1. Major polypeptide growth factors in bone development

Growth factor family	No. known members	Representative members	Molecular weight (kDa)	Receptors	Osteogenic function	Rep. Ref.
TGF-β	3	TGF-β1 TGF-β2 TGF-β3	25	*Serine/threonine receptors* TGF-βRI TGF-βRII	Stimulate proliferation of osteoblast precursors Increase osteoblast chemotaxis, Inhibit continued osteoblast differentiation *in vitro*	(70, 86)
BMPs	20	BMP-2 BMP-3 BMP-4 BMP-5 BMP-7	30–38	*Serine/threonine receptors* BMPR-IA (ALK-3) BMPR-IB (ALK-6) BMPR-II ActRIA (ALK-2)* ActR-II* ActR-IIB*	Induce ectopic bone formation Drive endochondral ossification Promote osteoblast differentiation Stimulate proliferation of osteoblasts Inhibit matrix synthesis	(16, 125)
IGF	3	Insulin IGF-I IGF-II	7.6	*Tyrosine kinase receptors* IGFR-IA IGFR-IB	Exerts significant proliferative effects on osteoblasts Local regulator of bone turnover	(52, 65)
FGF	22	FGF-1 FGF-2 FGF-3 FGF-8	17–34	*Tyrosine kinase receptors* FGFR-1b FGFR-1c FGFR-2b FGFR-2c FGFR-3b FGFR-3c FGFR-4	Stimulates osteoblast proliferation Accelerates fracture healing	(79, 80)

ActRIA, activin receptor type IA; BMP, bone morphogenetic protein; BMPR, BMP receptor; FGF, fibroblast growth factor; FGFR, FGF receptor; IGF, insulin-like growth factor; IGFR, IGF receptor; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor.

*Non-BMP specific, also binds activins.

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PERIODONTOLOGY 2000

Regeneration of vascularized bone

Susan X. Hsiong & David J. Mooney

Table 2. Representative studies of tissue engineering constructs for bone regeneration

Osteoinductive factor	Cells	Scaffold vehicle	Conclusions	Rep. Ref.
None	rBMSC	PLG foam	PLG scaffold supports osteogenic differentiation *in vitro*	(45)
None	RCO	RGD-modified alginate	Preosteoblasts encapsulated in RGD-modified alginate hydrogel exhibited enhanced differentiation *in vitro* and *in vivo*	(3, 5)
None	Sheep BMSC	Coral	Scaffold and cell vehicle improved healing in a sheep critical defect model	(85)
rhBMP-2	None	Collagen sponge	Significant increase in bony union of the rhBMP-2-treated groups in a rabbit distraction osteogenesis model	(98)
rhBMP-2	None	RGD, MMP-modified PEG hydrogel	Enhanced bone repair in a rat cranial defect model	(62)
BMP-4 (plasmid DNA)	None	Porous PLG	Enhanced bone repair in a rat cranial defect model	(39)
rhBMP-2 (adenoviral-mediated gene delivery)	hBMSC	Porous PLA	Bone matrix production and mineralization observed in *in vivo* diffusion chamber	(38)
rhTGF-β3 rhBMP-2	hBMSC	Degradable RGD-modified alginate	Degradable scaffold and dual growth factor delivery allow physiologic doses of growth factor for bone regeneration	(109)

HA, hydroxyapatite; hBMSC, human bone marrow stromal cells; MMP, matrix metalloproteinase; OP-1, osteogenic protein 1 (also BMP-7); PEG, poly(ethylene glycol); PLA, poly(lactide); PLG, poly(lactide-co-glycolide); rBMSC, rat bone marrow stromal cells; RCO, rat calvarial osteoblasts; rhBMP, recombinant human bone morphogenetic protein; RGD, arginine-glycine-aspartic acid; TGF-β3, transforming growth factor-β3.

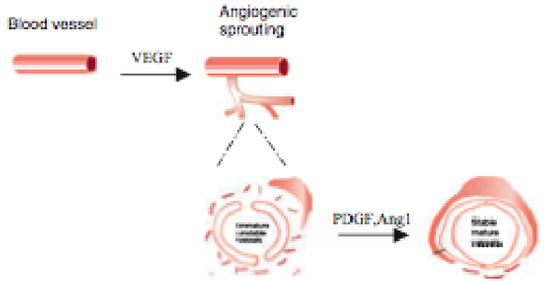

Fig. 4. Blood vessel formation via angiogenesis. Vascular endothelial growth factor (VEGF) stimulates new blood vessels to sprout from existing blood vessels, but the new vessels are immature and lack associated pericytes or smooth muscle cells. Angiopoietin 1 (Ang 1) and platelet-derived growth factor (PDGF) promote the association of pericytes and smooth muscle cells with the nascent vessels, which leads to the formation of stable, mature vessels. Reprinted from Yancopoulos et al. (128), with permission from Nature.

Table 3. Representative approaches to enhance bone formation by promoting vascularization

Angiogenic factor	Cells	Vehicle/mode of delivery	Model	Ref.
None	ECs hBMSC	Porous PLG	Rat subcutaneous implantation	(129)
VEGF (adenovirus)	None	Intramuscular injection around bone defect	Rat femur defect	(115)
None	rBMSC	Coral	Rat intramuscular implantation with or without vascular pedicle	(82)
VEGF	None	Biomineral-coated PLG scaffold	Rat cranial defect	(73)
VEGF BMP-4 (plasmid DNA)	hBMSC	Porous PLG	Mouse subcutaneous implantation	(40)
VEGF* BMP-4*	hMDSC	Gelfoam scaffold	Mouse cranial defect Mouse femur defect	(83)

ECs, endothelial cells; hBMSC, human bone marrow stromal cells; hMDSCs, human muscle derived stem cells; HOBs, human osteoblasts; HUVECs, human umbilical vein endothelial cells; PLG, poly(lactide-co-glycolide); rBMSC, rat bone marrow stromal cells, VEGF, vascular endothelial growth factor.
*Retroviral-mediated gene delivery.

Interface focus

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doi:10.1098/rsif.2008.0448.focus
Published online 4 March 2009

REVIEW

Biomaterial technology for tissue engineering applications

Yasuhiko Tabata*

Table 1. Biodegradable polymers used for tissue engineering of cell scaffold and biosignalling molecule release.

synthetic polymers	natural polymers
poly(L-lactic acid) (PLLA)	collagen
poly(glycolic acid) (PGA)	gelatin
poly(e-caprolactone) (PCA)	fibrin
copoly(LL-GA)	
copoly(LL-CA)	hyaluronic acid[a]
copoly(LLA-ethylene glycol (EG))	alginate[a]
copoly(fumarate-EG)	chitosan, chitin

[a]There are no enzymes in the body to directly degrade these polymers. They are washed out by body fluids to disappear from the implanted site.

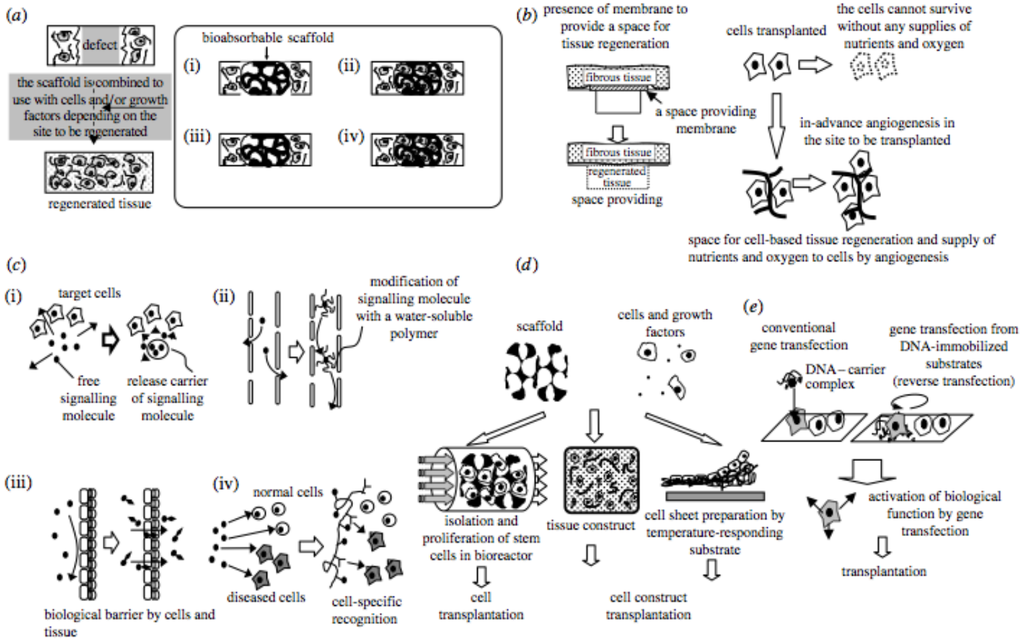

Figure 2. Role of biomaterials in tissue engineering-based regeneration therapy. (*a*) Biomaterials for cell scaffold to induce *in vivo* tissue regeneration. Bioabsorbable scaffold: (i) without cells and growth factors, (ii) with cells, (iii) with growth factors, (iv) with cells and growth factors. (*b*) Biomaterials to protect a space and induce angiogenesis for *in vivo* tissue regeneration. (*c*) Biomaterials for DDS of biosignalling molecules (growth factors and genes): (i) controlled release of signalling molecule, (ii) prolongation of signalling molecule lifetime, (iii) absorption acceleration of signalling molecule, (iv) signalling molecule targeting. (*d*) Biomaterials for *in vitro* cell manipulation to obtain cells and cell constructs for transplantation. (*e*) Biomaterials for engineering biological functions of cells.

Biomaterials play a key role in designing and creating substitutes for ECM and the drug delivery system (DDS) of biosignalling molecules to enhance their biological activities. In addition to therapeutic applications, biomaterials are also useful in the progress of research and development of stem cell biology and medicine.

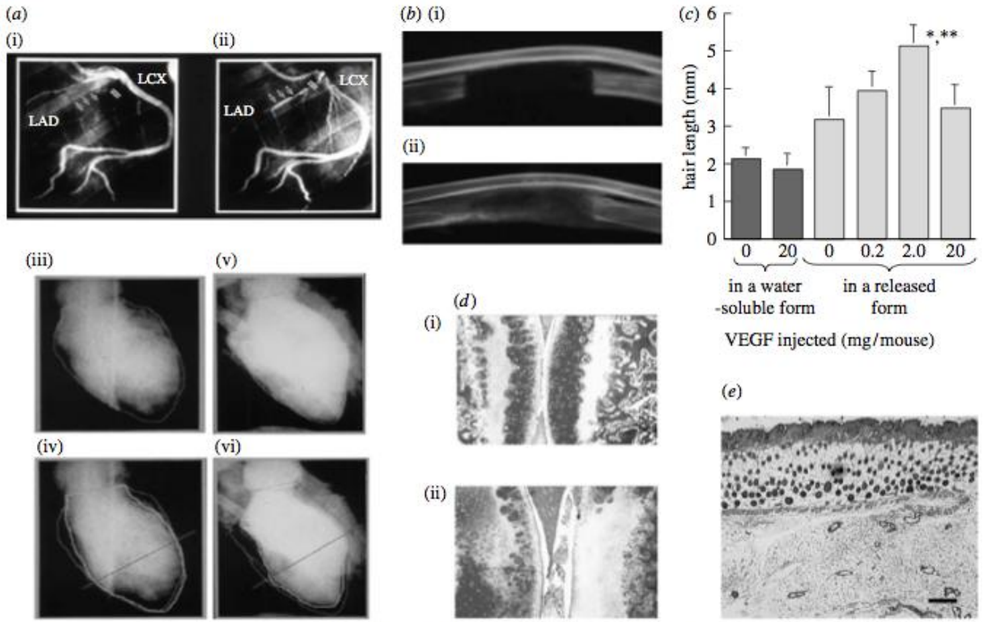

Figure 3. Examples of tissue regeneration with biodegradable hydrogels for growth factor release. (*a*) Regeneration of corona artery: (i) bFGF solution, and (iii) diastole, (iv) systole; (ii) gelatin microspheres incorporating bFGF, and (v) diastol (vi) systole (LAD, left arterior descending coronary artery; LCX, left circumflex coronary artery). (*b*) Bone regeneratio (i) BMP-2 solution, (ii) hydrogel incorporating BMP-2. (*c*) Promotion of hair shaft elongation ($*p<0.05$ versus water-solub form; $**p<0.05$ versus other VEGF concentrations in a released form). (*d*) Articular cartilage regeneration: (i) CTGF solutio (ii) gelatin microspheres incorporating CTGF. (*e*) Fat tissue regeneration: gelatin microspheres incorporating bFG Currently, approximately 360 collaborations with the release technology of various growth factors are being performed b clinicians and researchers.

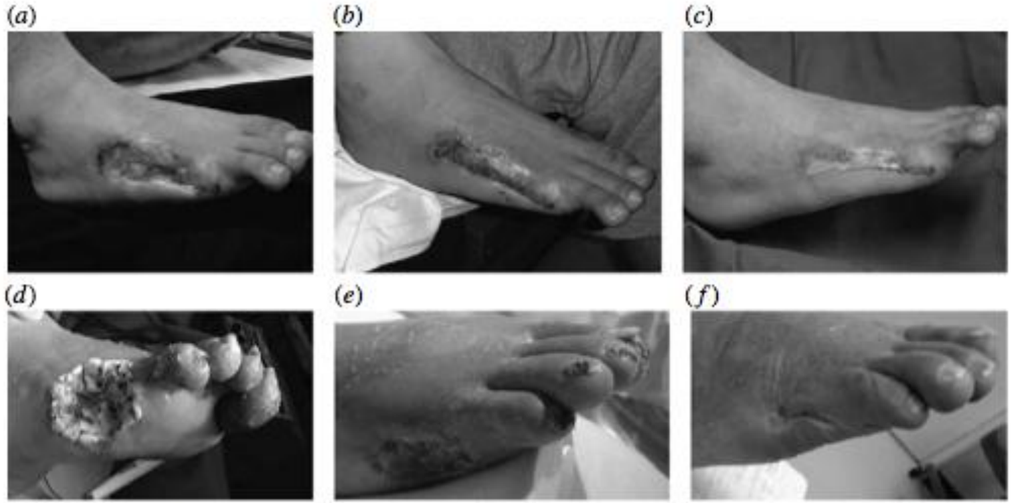

Figure 4. Examples of clinical tissue regeneration for ischaemic ASO and diabetic foot ulcer of intractable disease with biodegradable hydrogels for bFGF release. Intractable diseases could be repaired only by the intramuscular injection or implantation of hydrogel granules incorporating bFGF. bFGF was locally released over two weeks at the site injected to induce *in vivo* angiogenesis resulting in promoted wound healing. The first clinical case worldwide: (*a*) 27 years, male, (*b*) four weeks later, (*c*) 12 weeks later; (*d*) 73 years, female, (*e*) four weeks later, (*f*) 16 weeks later. Before treatment, the patients could not walk due to their severe pain. But angiogenic therapy allowed them to walk without any problem.

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Cartilage tissue engineering PLLA scaffold with surface immobilized collagen and basic fibroblast growth factor☆

Zuwei Ma, Changyou Gao*, Yihong Gong, Jiacong Shen

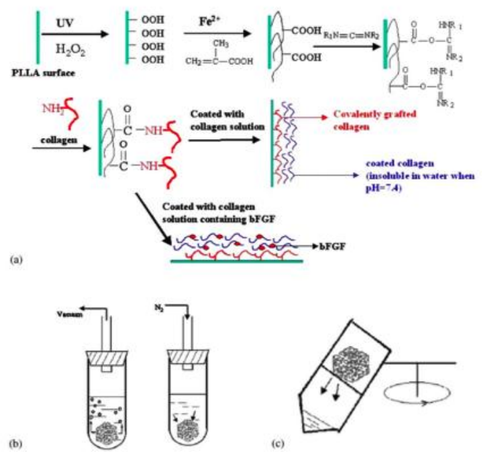


Fig. 1. Schematic representation of (a) the reaction scheme in the surface modification of PLLA, (b) the pressing of liquid reagent into PLLA scaffold through vacuum treatment and (c) the removal of liquid reagent out of the PLLA scaffold through centrifuging treatment.

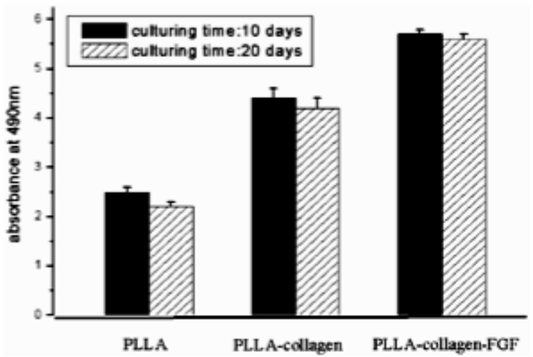
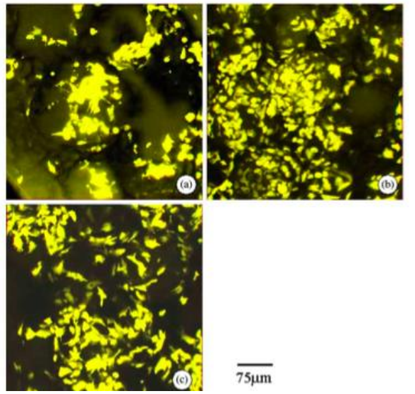


Fig. 4. MTT viability of chondrocytes cultured in the control and modified PLLA scaffolds. Cell seeding density is 600×10^4 cells/ml.



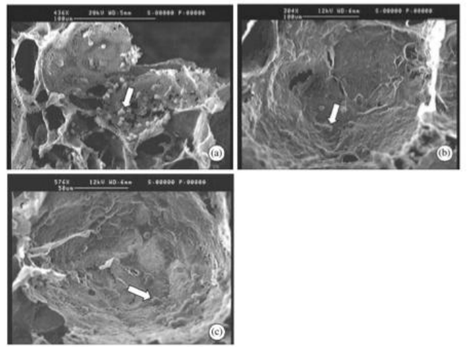


Fig. 6. SEM images of the chondrocytes in (a) the control PLLA scaffold, (b) PLLA–collagen scaffold and (c) PLLA–collagen–bFGF scaffold. Seeding density is 600×10^4 cells/ml. Culture time is 2 weeks. The arrows point to the cells.

RESEARCH REPORTS
Biological

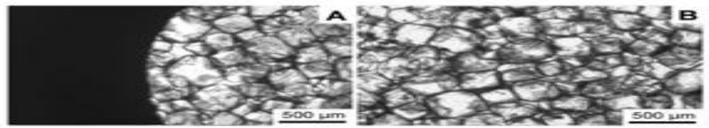
L. Casagrande[1,2], F.F. Demarco[2,3], Z. Zhang[2], F.B. Araujo[1], S. Shi[4], and J.E. Nör[2,5,6]*

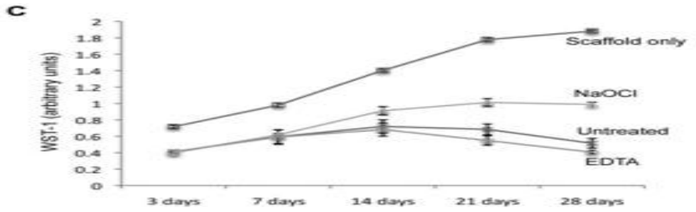
Dentin-derived BMP-2 and Odontoblast Differentiation

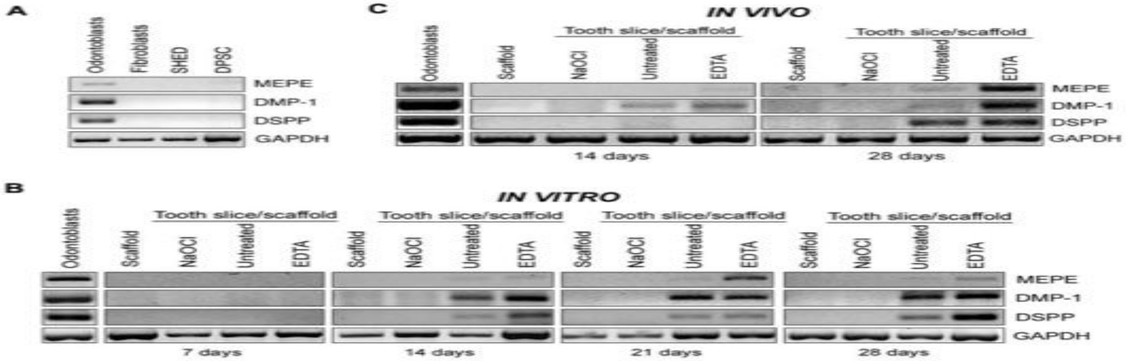
JDR - CURRENT ISSUE

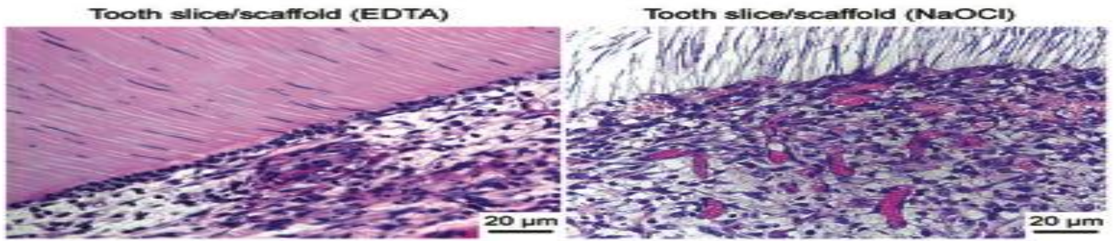
Dentin-derived BMP-2 and Odontoblastic Differentiation

Luciano Casagrande[1,2], Flávio F. Demarco[2,3], Zhaocheng Zhang[2], Fernando B. Araujo[1], Songtao Shi[4], Jacques E. Nör[2,5,6]



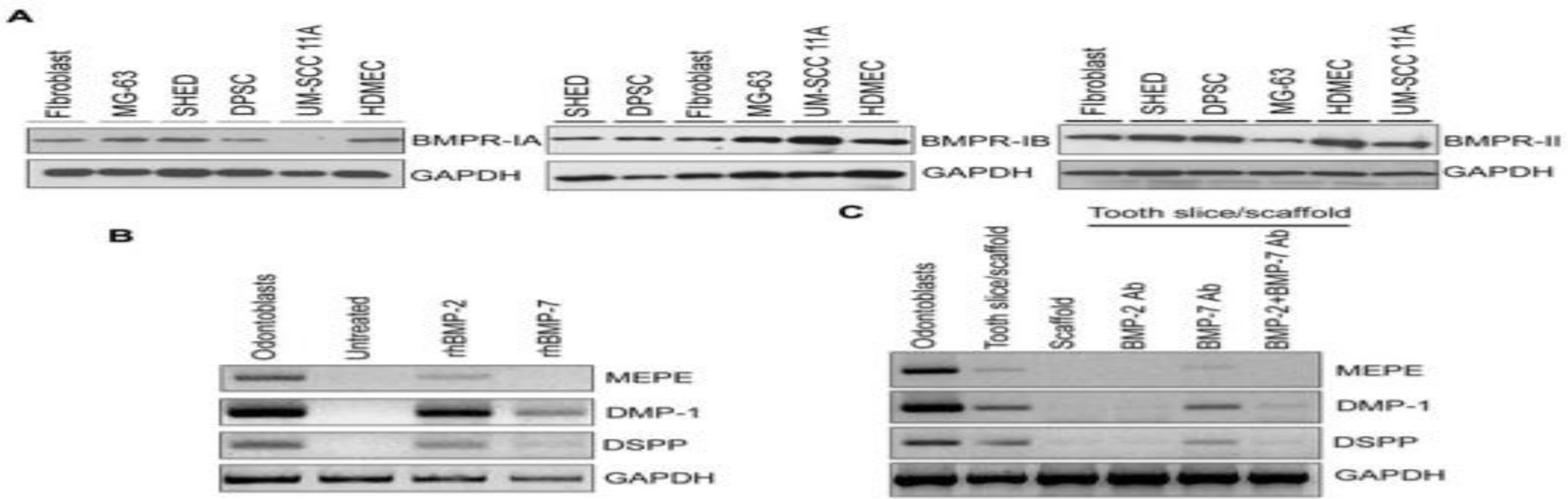






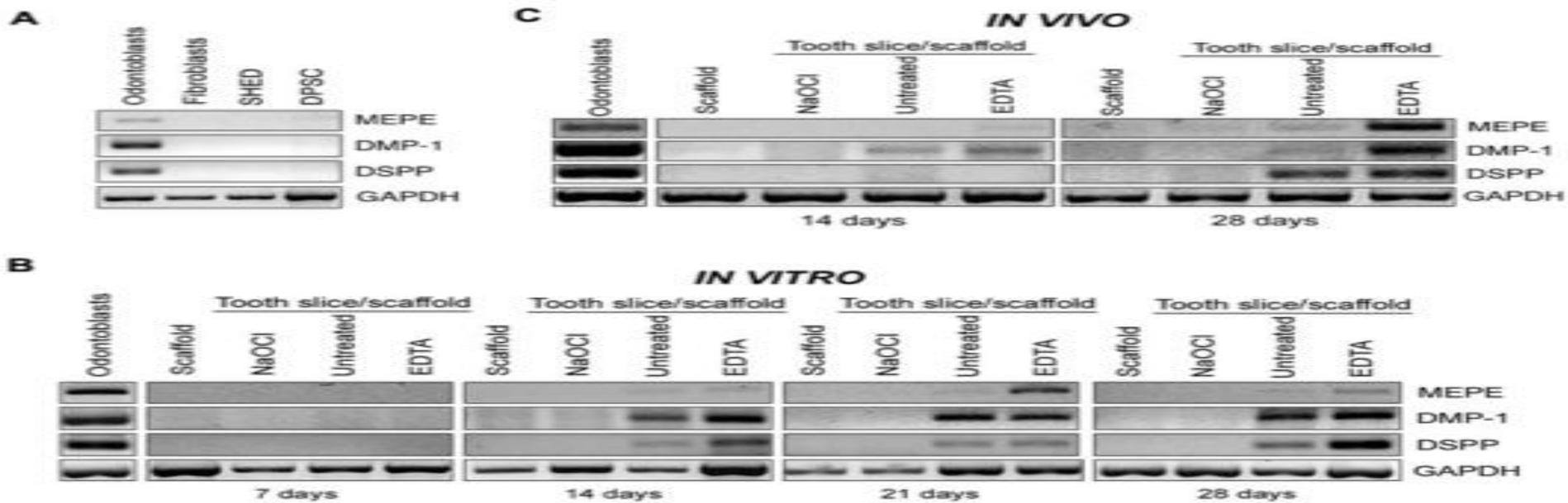
Dentin-derived BMP-2 and Odontoblastic Differentiation

Luciano Casagrande[1,2], Flávio F. Demarco[2,3], Zhaocheng Zhang[2], Fernando B. Araujo[1], Songtao Shi[4], Jacques E. Nör[2,5,6]



Dentin-derived BMP-2 and Odontoblastic Differentiation

Luciano Casagrande[1,2], Flávio F. Demarco[2,3], Zhaocheng Zhang[2], Fernando B. Araujo[1], Songtao Shi[4], Jacques E. Nör[2,5,6]

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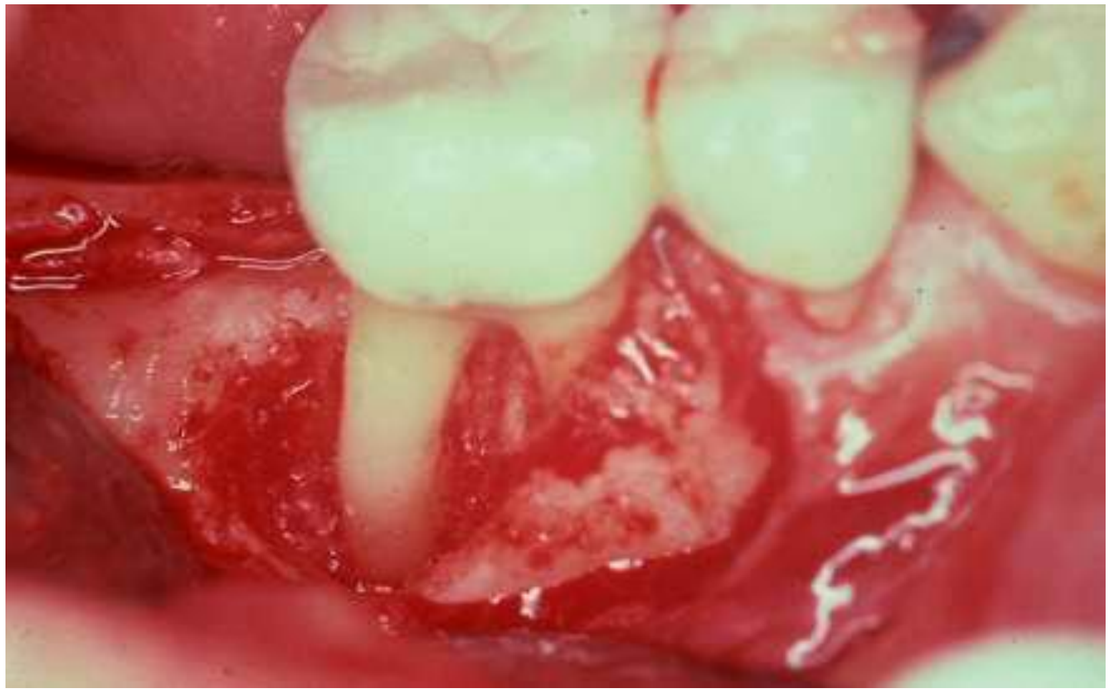
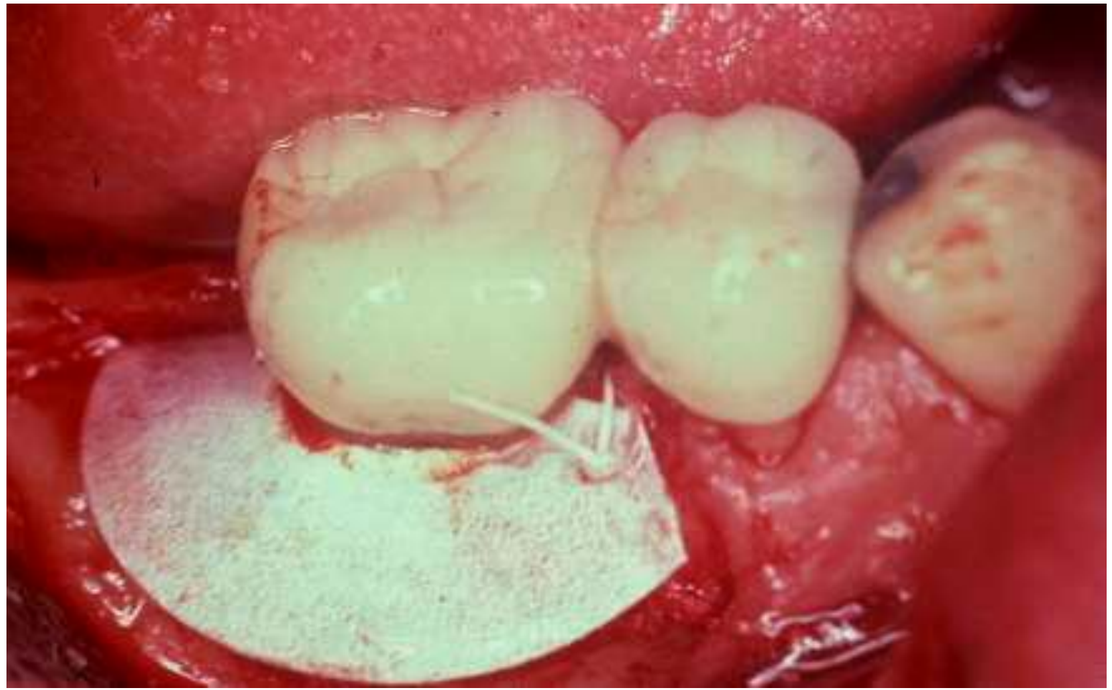
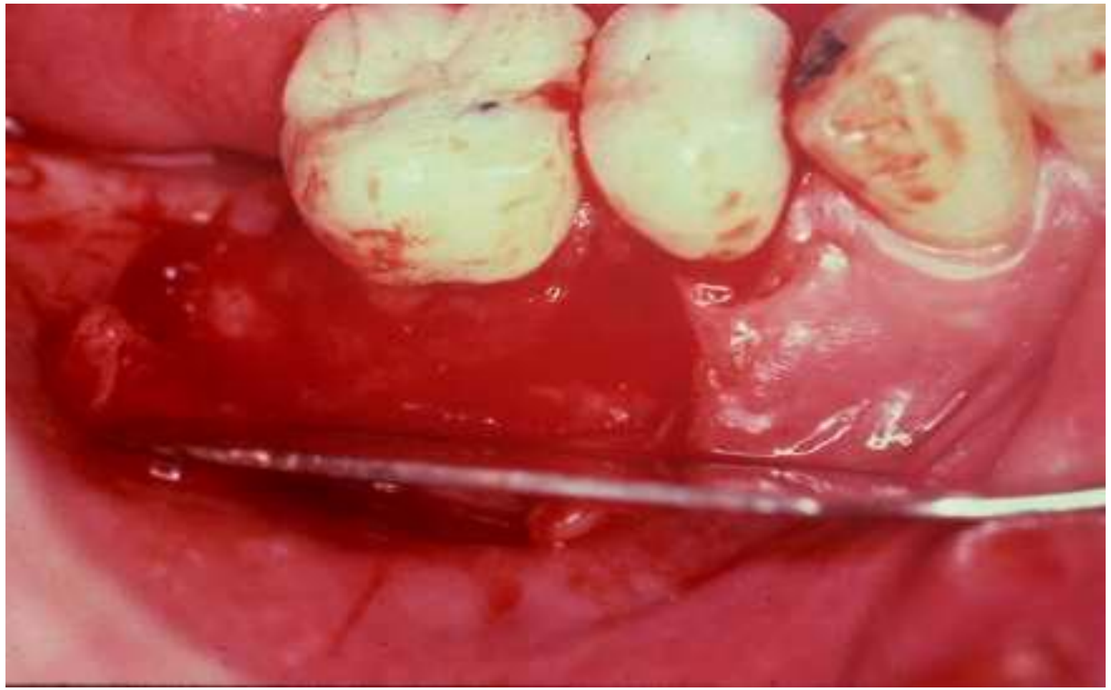
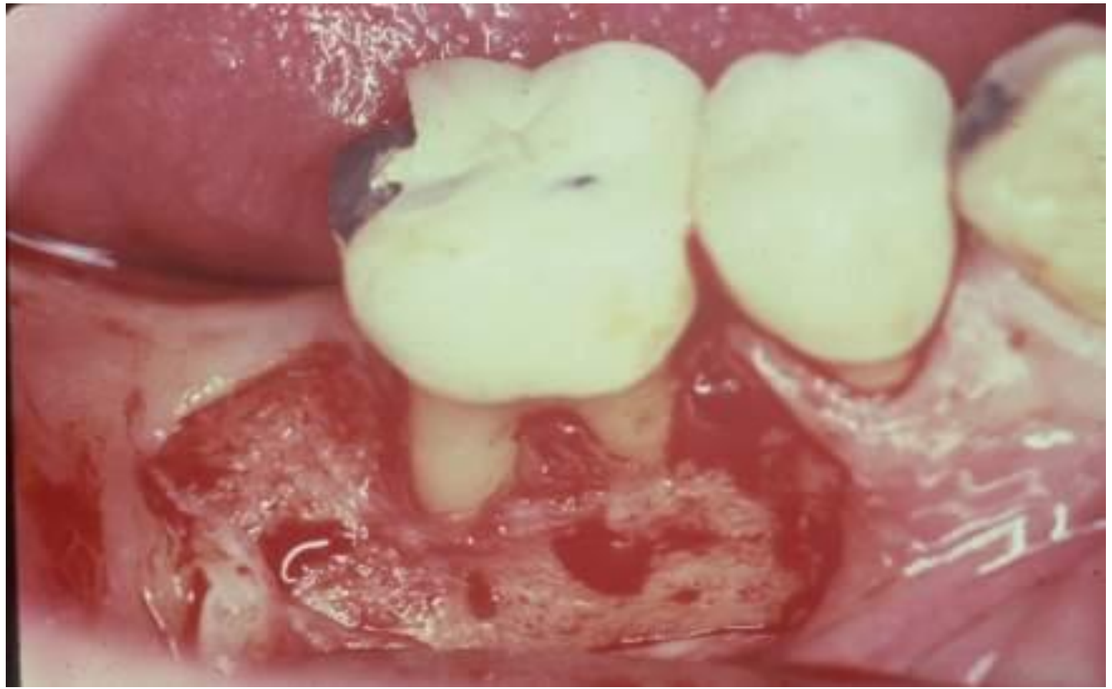
Item No. SN2BS $285.00 per box of 3 ($95.00 each)	Item No. SW2BS $315.00 per box of 3 ($105.00 each)	Item No. WA2BS $360.00 per box of 3 ($120.00 each)	









Bio-Active Molecules

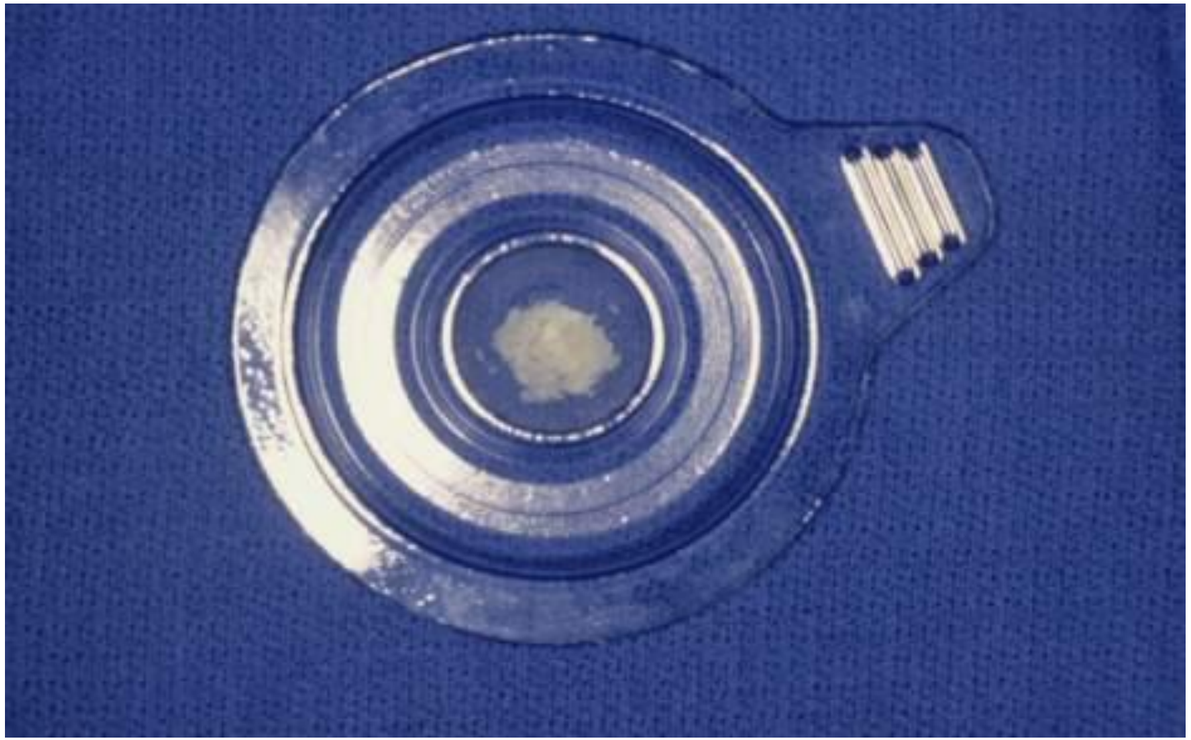
Bone Morphogenetic Proteins (B.M.P.)

BMPs need carrier to get effective bone initiation.

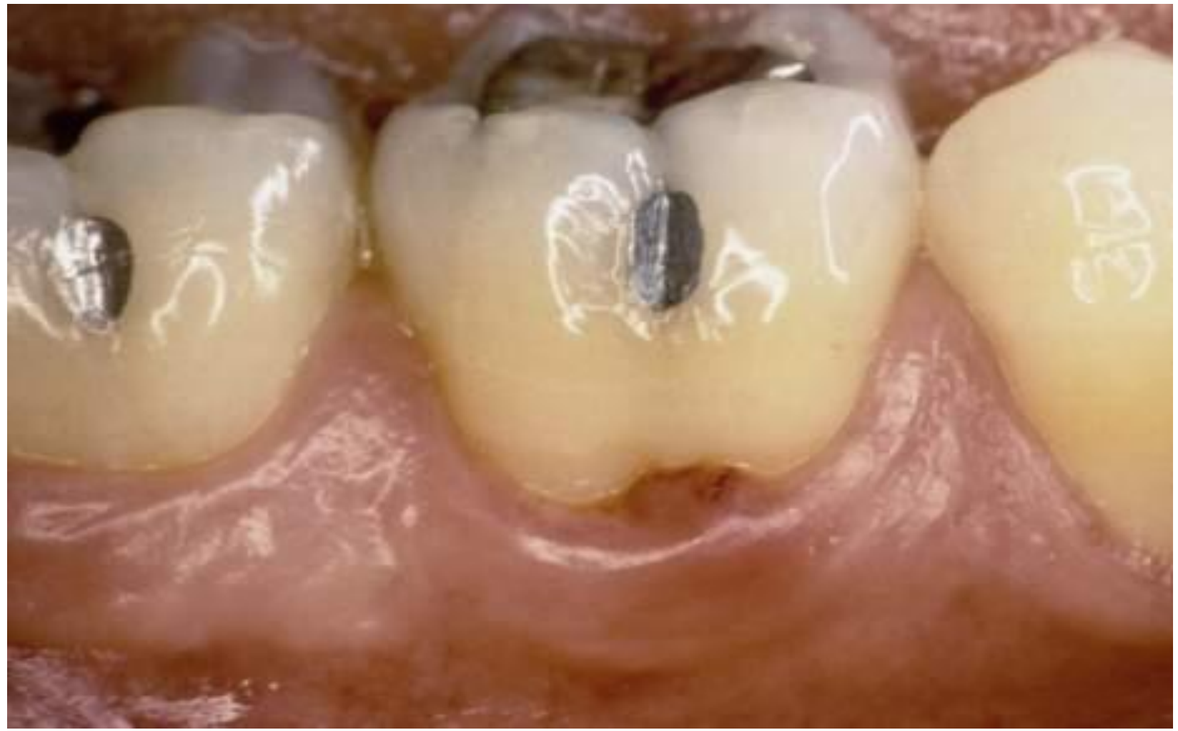
Ideal carrier still not found.

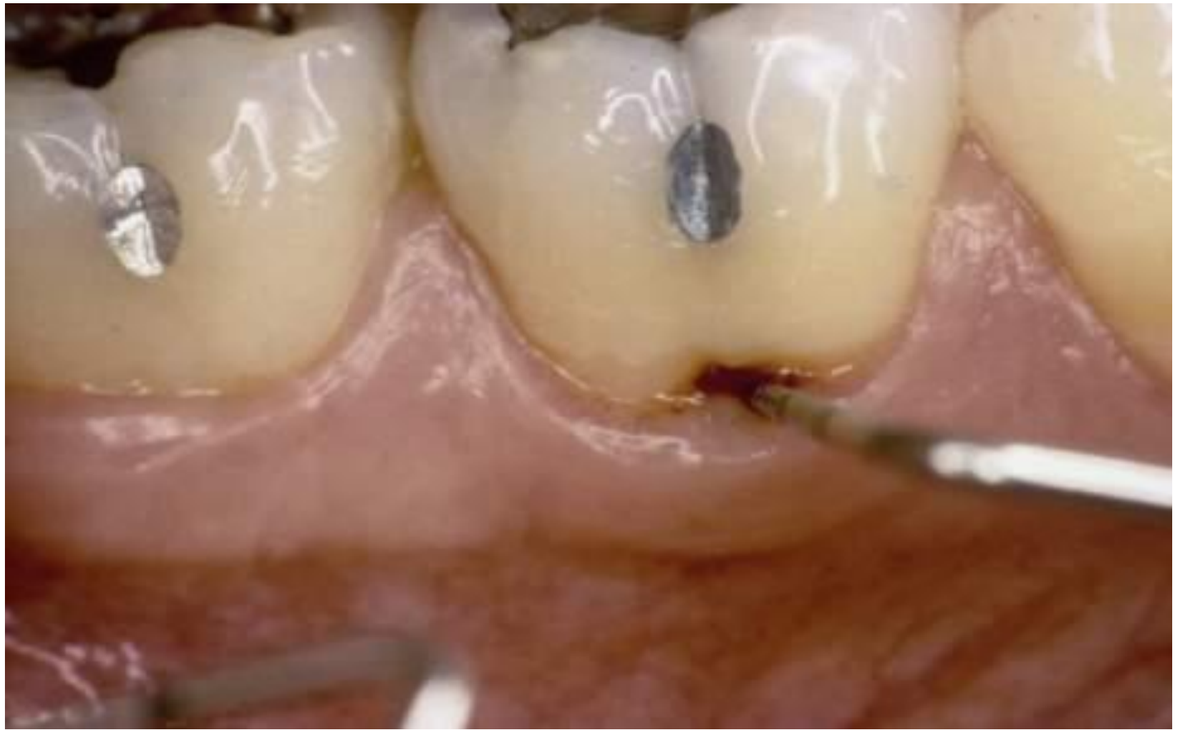
Carriers:

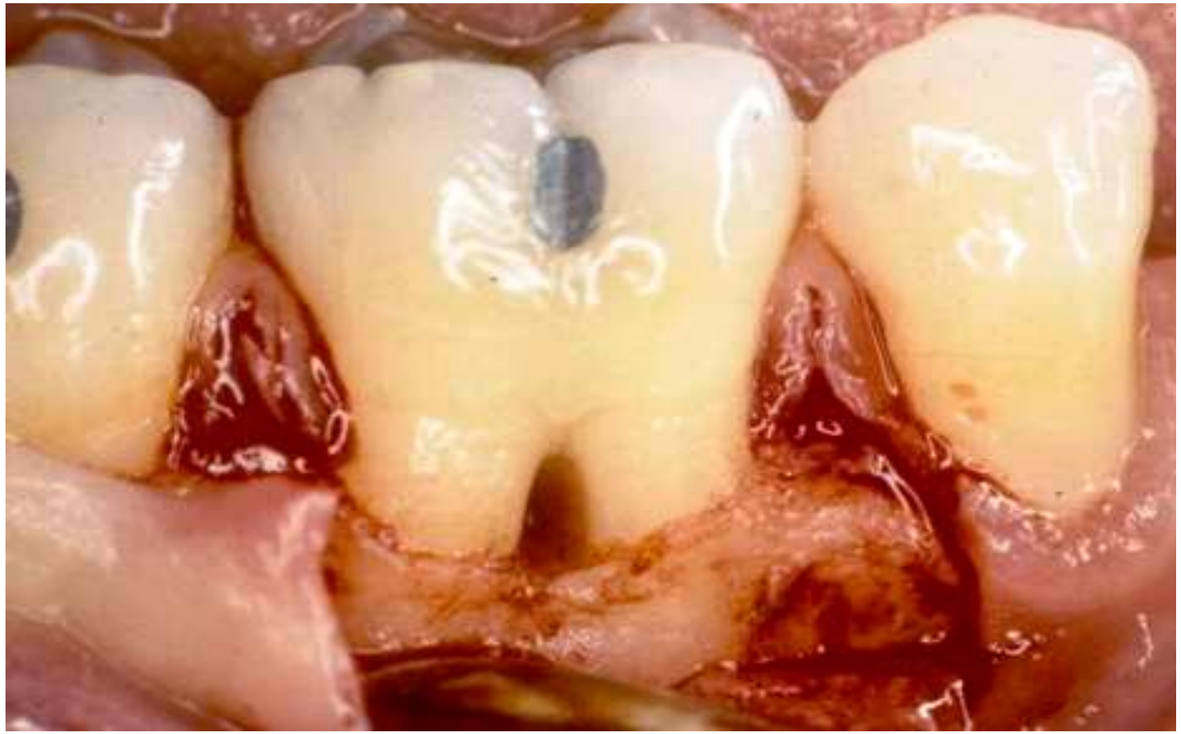
- Demineralized Bone Matrix
- Collagen
- Resorbable polymers
- Calcium phosphate materials

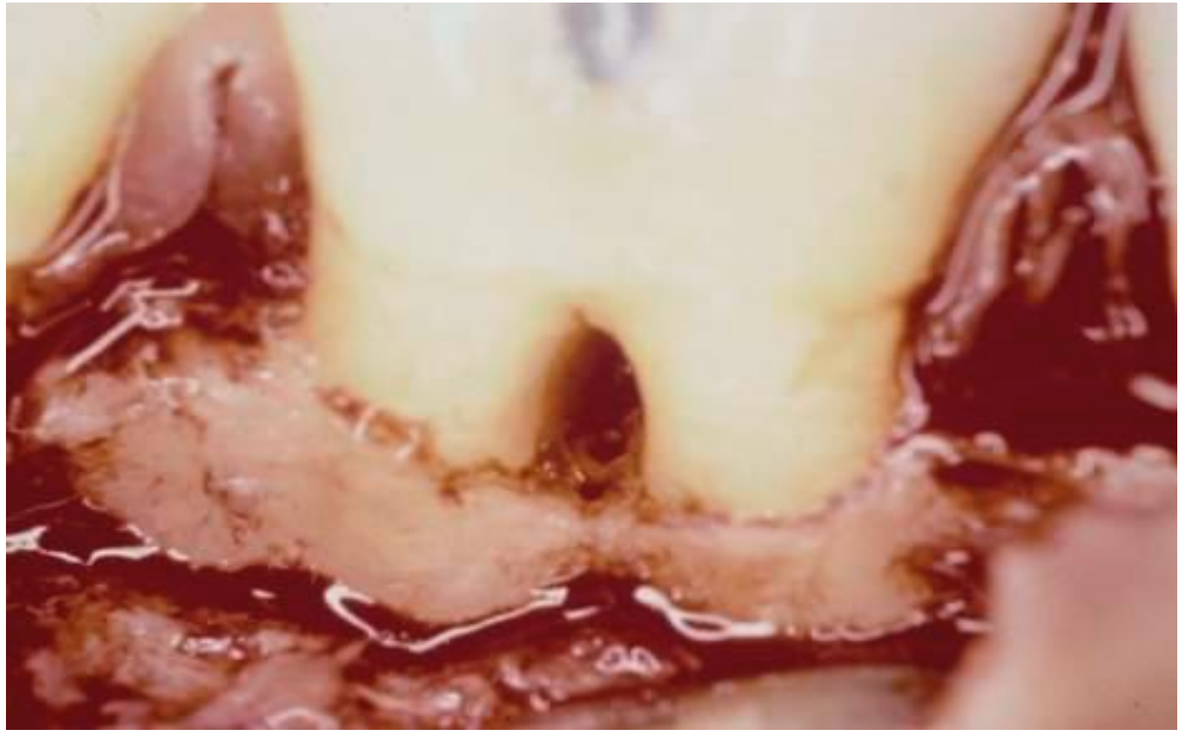


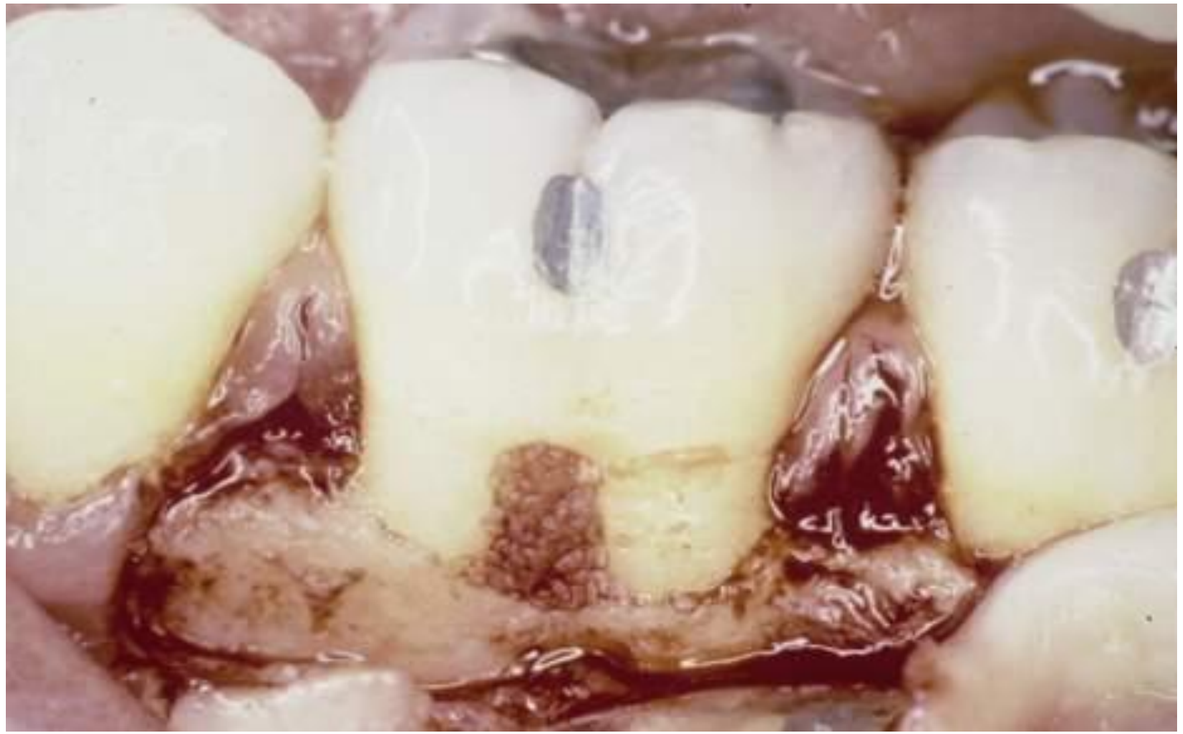
Highest concentrations of BMP gave best clinical results

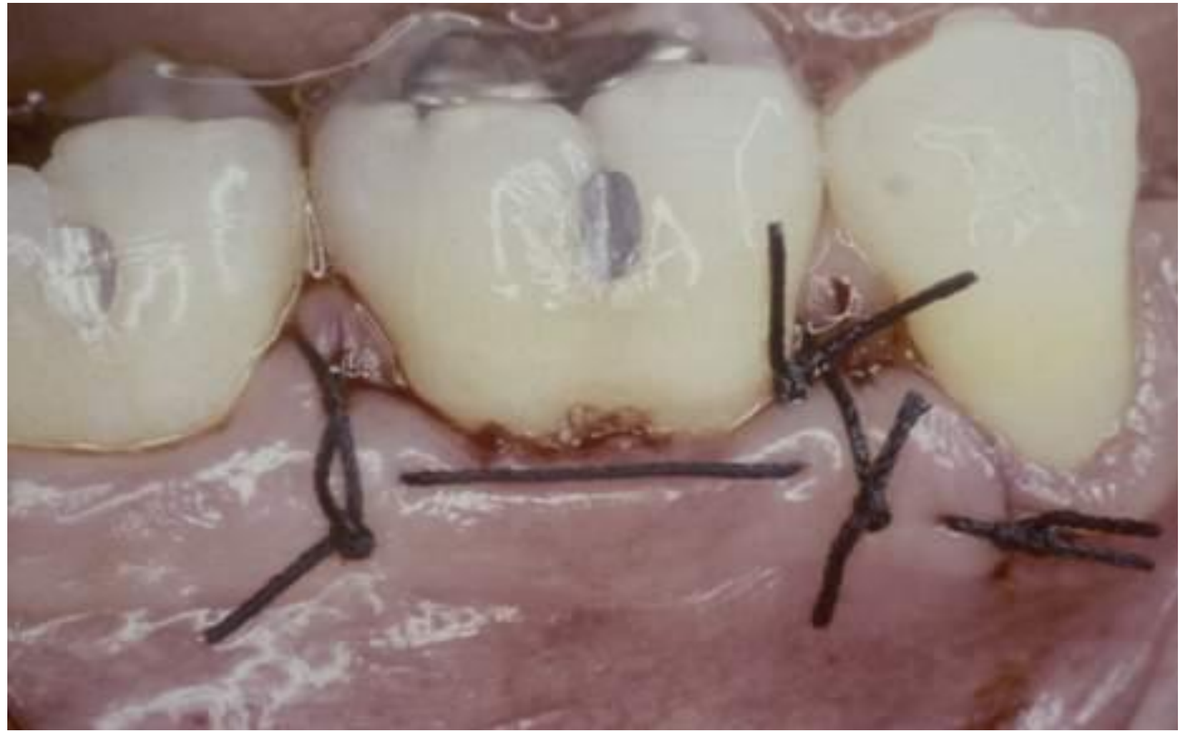


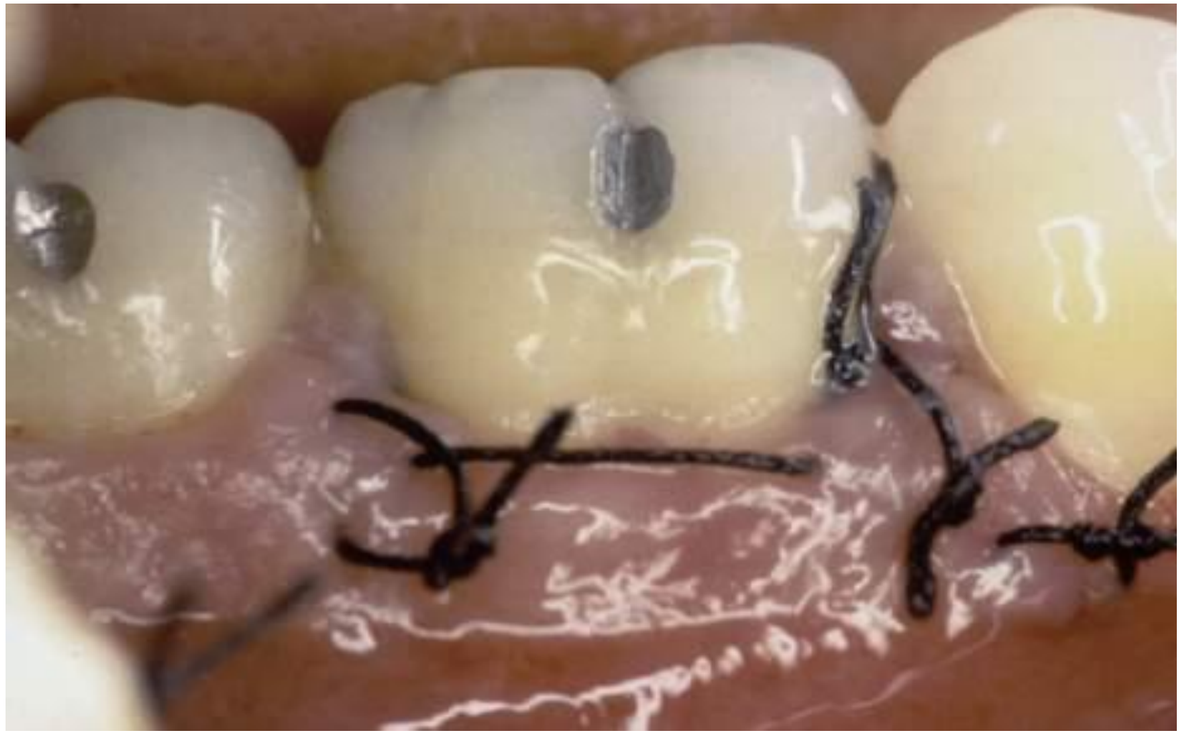


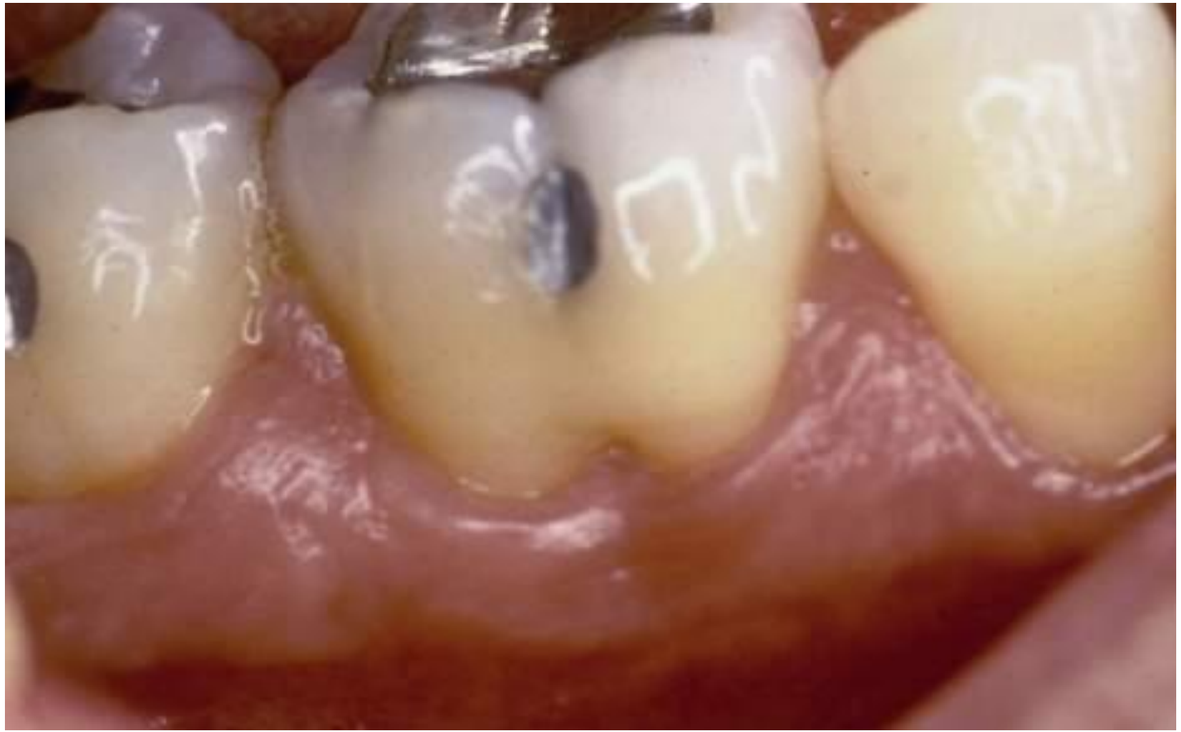


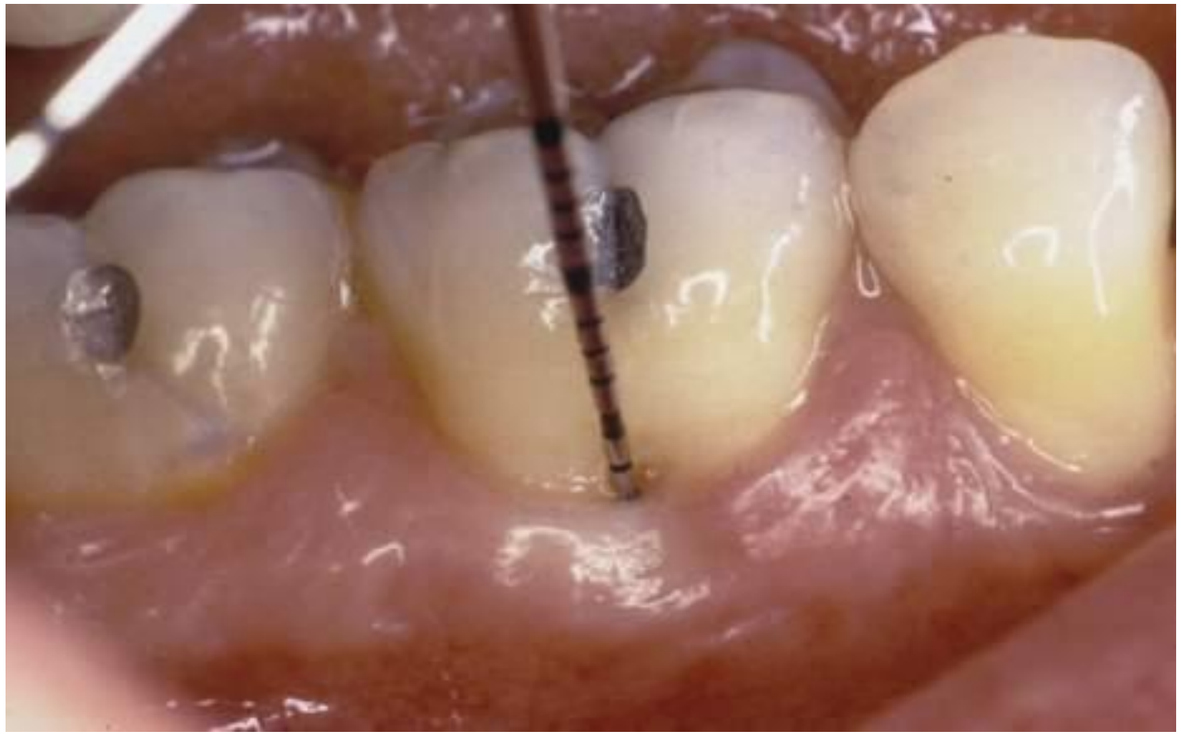


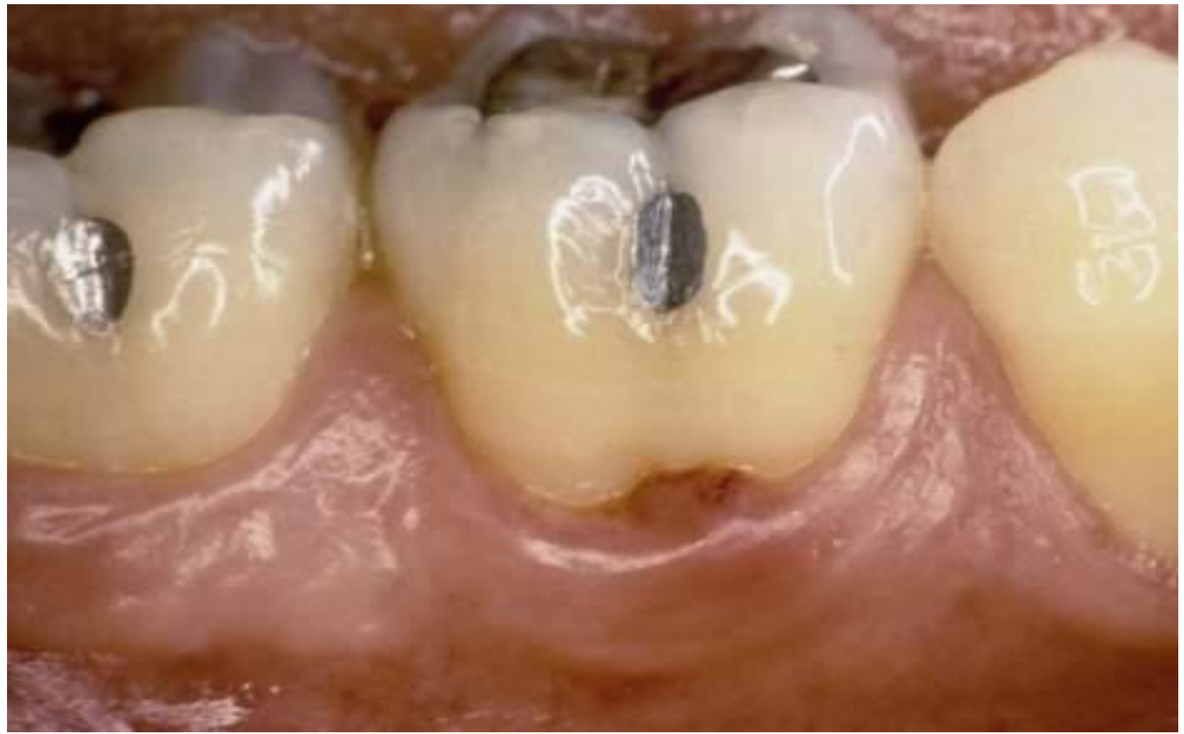












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Nano-fibrous scaffold for controlled delivery of recombinant human PDGF-BB

Guobao Wei [a], Qiming Jin [b], William V. Giannobile [a,b], Peter X. Ma [a,c,d,*]

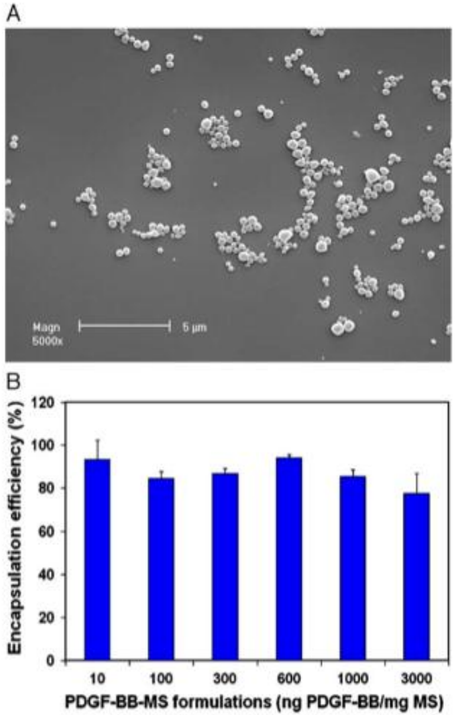

Fig. 1. Characterization of PLGA50-6.5K microspheres (MS). (A) Scanning electron micrograph of FITC-BSA containing PLGA50-6.5K microspheres; (B) encapsulation efficiency of PDGF-BB in PLGA50-6.5K microspheres with varying loading amount from 10 to 3000 ng/mg MS.

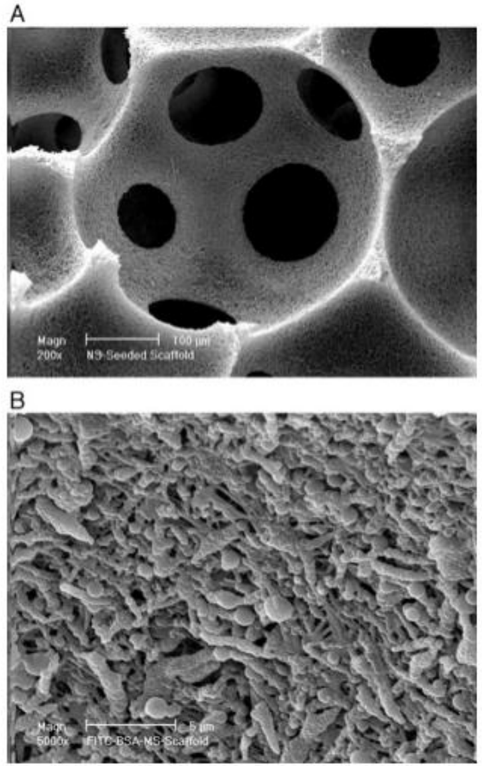

Fig. 3. Scanning electron micrographs of PLLA nano-fibrous scaffolds after PLGA50-6.5K microsphere incorporation using post-seeding method. (A) Low magnification at 200×; and (B) high magnification at 5000×.

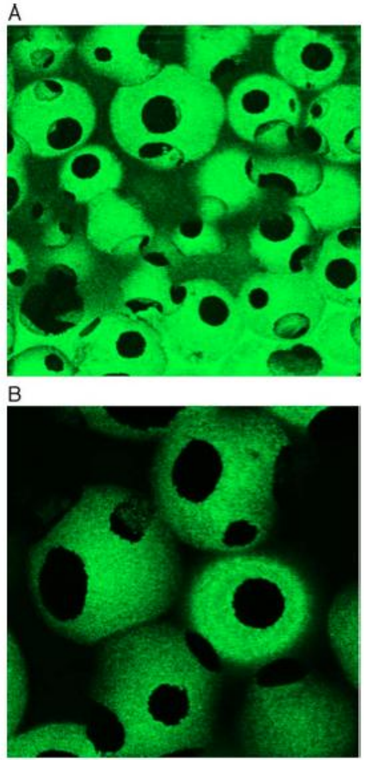

Fig. 4. Laser scanning confocal microscopy (LSCM) of cross section through PLLA nano-fibrous scaffold containing FITC-BSA microspheres incorporated, revealing uniform microsphere distribution throughout the scaffold. Original magnification is (A) 100×; and (B) 200×.

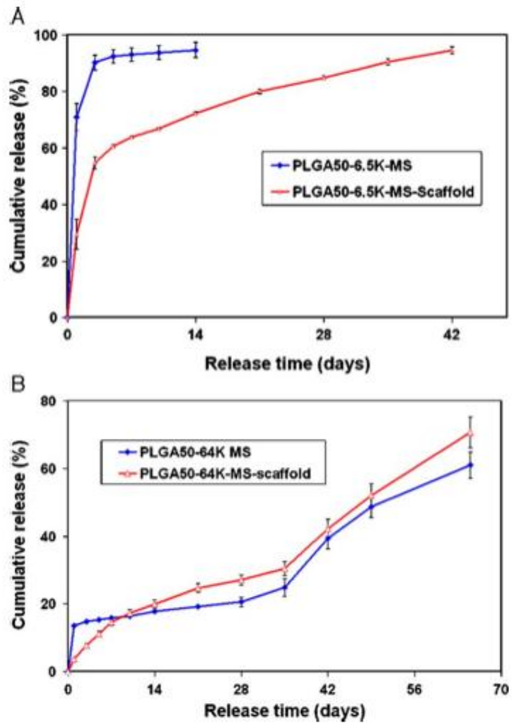

Fig. 6. In vitro release kinetics of PDGF-BB from microsphere-incorporated PLLA scaffolds in PBS/SDS. (A) PLGA50-6.5K-MS-scaffold; and (B) PLGA50-64K-MS-scaffold. Each data point represents an average±standard deviation (n=3). The experiment was repeated twice.


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