Biological mediators and periodontal healing

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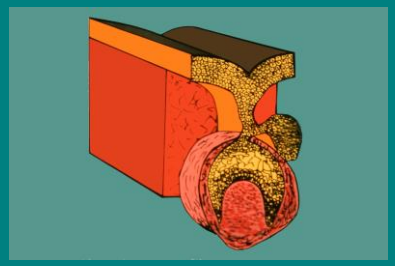
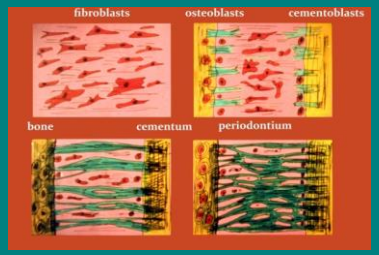
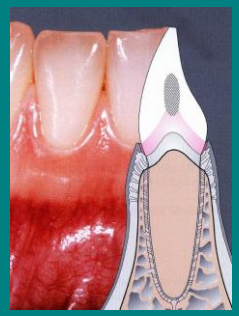
The therapeutic objective of periodontal treatment is the regeneration of the specific periodontal tissues.

(Gottlow, Nyman, Karring, 1992; Spector, 1994)







Cementum
Periodontal ligament
Alveolar bone

develop together

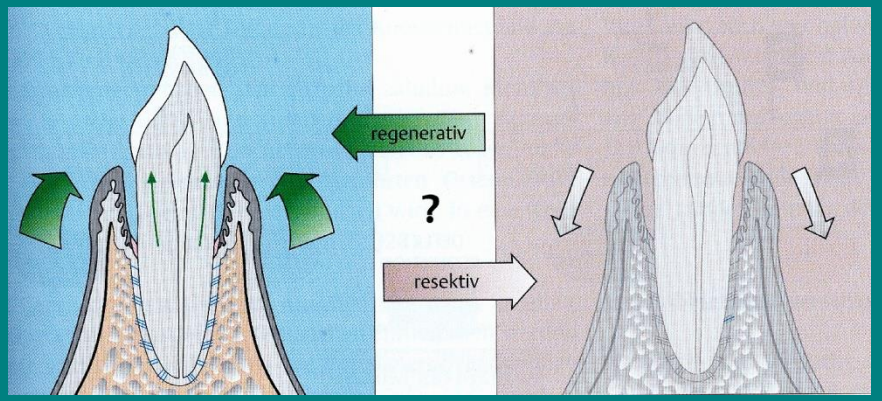
(Ten Cate, 1975)

- their regeneration is also associated

(Hammarström, Heijl, 1997)

Periodontal surgery

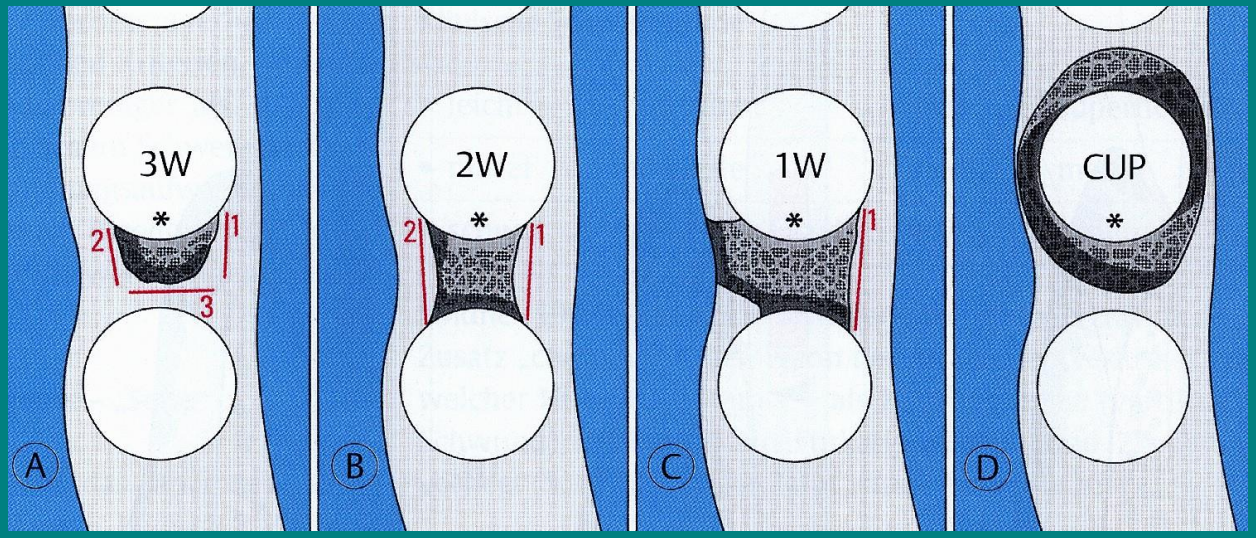
Regenerative periodontal techniques



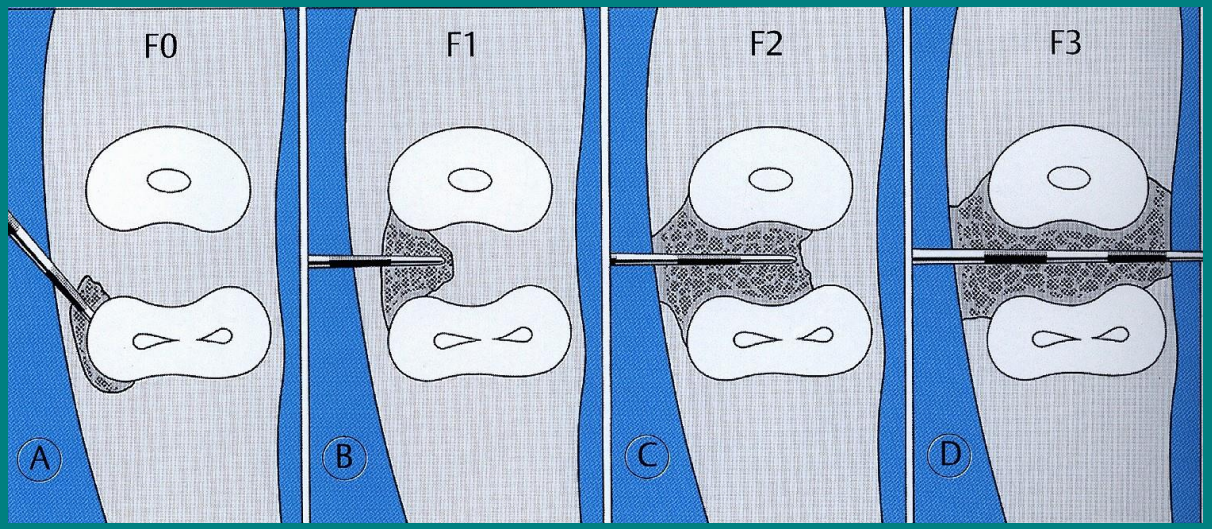
1. Membrane techniques
2. Enamel matrix derivatives
3. Combined methods
4. Growth factors, stem cells…

…nanotechnology… → ?

Intrabony defects



Furcation lesions



Biological mediators

- Enamel-matrix derivatives/proteins

  EMD/EMP

- Growth Factors, Growth and Differentiation Factors

  GFs/GDFs

EMD

Emdogain®

/Manufacturer: Straumann Biologics, Switzerland/

Enamel matrix proteins are involved in the formation of cementum *(Slavkin, Boyde 1975)* **and they have the potential to induce regeneration of accellular cementum on root dentin surface.** *(Hammarström, 1997)*

The development and approval of enamel matrix proteins for periodontal regeneration: a new era in periodontal regenerative therapy.

(Caton, 1997)

Healing:

- residual adherent and non-dissolvable enamel matrix protein layer remains on the conditioned root surface (barrier membrane role)

→ appearence of **cementoblasts**

- the *regenerating periodontal ligament* has a marked osteoinductive activity

→ **alveolar bony regeneration**

The biochemical environment at the root surface after using EMD-s may also prevent the epithelial down-growth.

(Hammarstörm, Heijl,1997)

Emdogain®

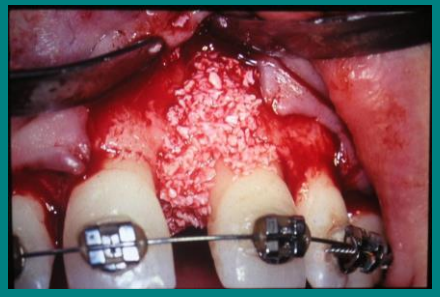
Application:

-used alone:

- surgery of bone defects
- mucogingival surgery

-in combination with bone grafts:

- vertical bone defects
- furcation lesions, combined defects



Enamel – matrix proteins

: 90% : - amelogenines

: 10%: - proline-containing non-amelogenines
- „tuftelin”
- „tuft protein”
- serum proteins
- syalo - protein

+ ameloblastin, amelin

EMD

Amelogenin

EMP

- a protein with an important role in the development of teeth and supporting structures

- produced ONLY during the ontogenesis of the tooth and the periodontium!

Emdogain

Clean root surface

The organism „thinks” that a new supporting structure is developing

Enamel – matrix proteins

- regulate the development, growth and maturation of hydroxyapatite crystals of the enamel

*

amelogenin-fraction

- helps the acellular cementogenesis (other function than amelogenesis)

Periodontology

Emdogain gel

12-24 hours

carrier agent

(propylene-glycol alginate)

leaves

Coagulum

+

insoluble and adherent protein layer on the root surface

*

Coagulum

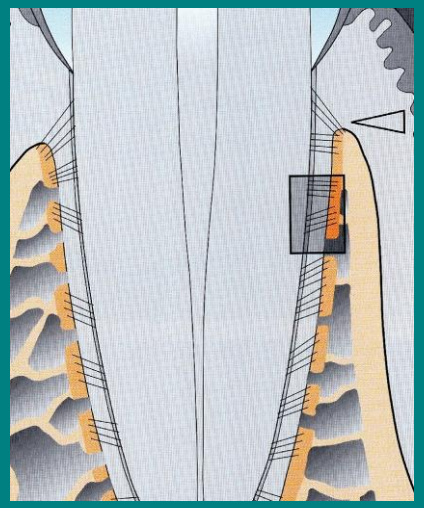
2-3 weeks

well organized granulation tissue

cementoblast activity

Emdogain®

- capacitate the root surface for new cement formation
- helps the redevelopment of supporting periodontal tissues:



- acellular cementum
- functional peridontal ligaments
- alveolar bone

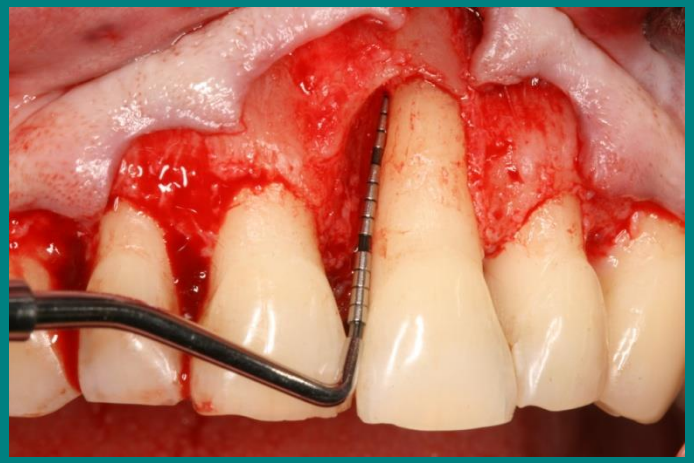
The Emdogain® promotes the natural biological reaction of the body

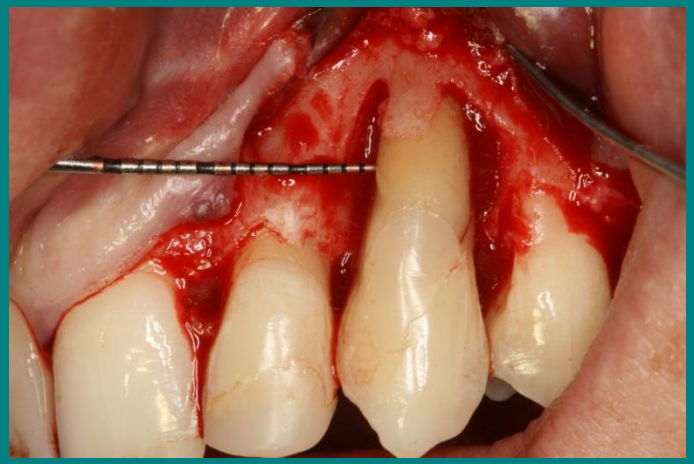
Growth and differentiation factors (GDFs)

- found in bone, cementum and healing wound tissues:

- platelet derived growth factor **(PDGF)**
- vascular endothelial growth factor **(VEGF)**
- transforming growth factors **(TGF - α and β)**
- acidic and basic fibroblast growth factors **(a- and b FGF)**
- epidermal growth factor **(EGF)**
- insulin like growth factors **(IGF – I and II)**
- cementum derived growth factor **(CDGF**)
- parathyroid hormone – related protein **(PTHrP)**
- bone morphogenetic proteins **(BMPs 1-12)**

GFs → Periodontology





Polypeptide growth factors:

natural biological mediators which play a critical role in the stimulation and regulation of the wound healing process.

The objective of their administration in treatment of periodontitis is to enhance the normal wound healing response which may be insufficient for the regeneration of all attachment structures.

(Position Paper of the American Academy of Periodontology, 1996; res. 2007)

GFs

Wound healing

Periodontology

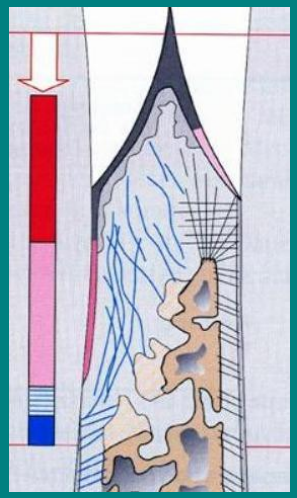
Gingival epithelial cells, gingival fibroblasts and periodontal ligament (PDL) fibroblasts are the most important cells in the soft tissue repair process for new attachment.

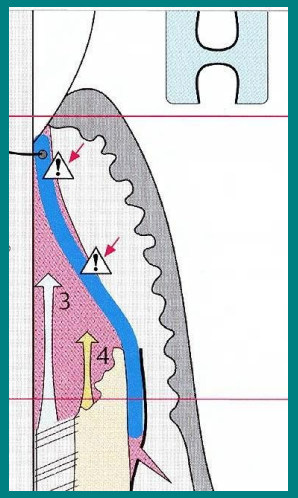
For periodontal regeneration, the coronal re-establishment of the PDL is required together with corresponding cementum and supporting alveolar bone. *(PP of the AAP)*

GFs

Regeneration

In the organizing stage of a clot, growth factors play a major role in healing and osseous regeneration phenomena. *(Anitua E.)*





PRP

Platelet – Rich Plasma

Concentration of human platelets by centrifugation
⟶ human autologous platelets of 338%

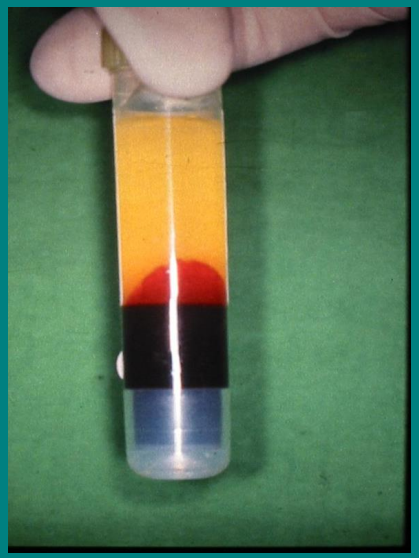
an autologous source of :

- platelet-derived growth factor (PDGF)
- transforming growth factor beta (TGF-β)

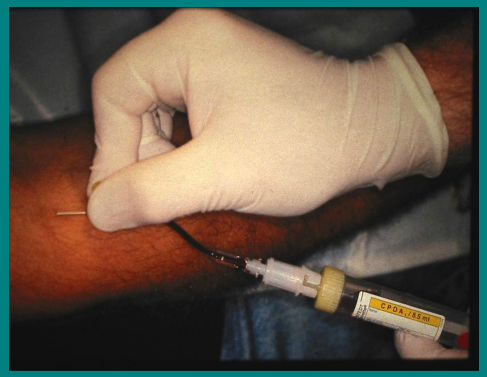
(Marx R. E. , 1998)

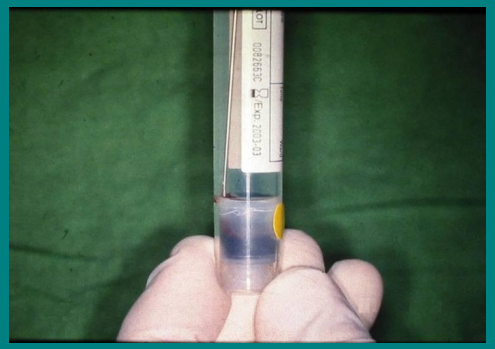
Properties:

- nontoxic
- nonimmunoreactive
- accelerates existing wound - healing pathways

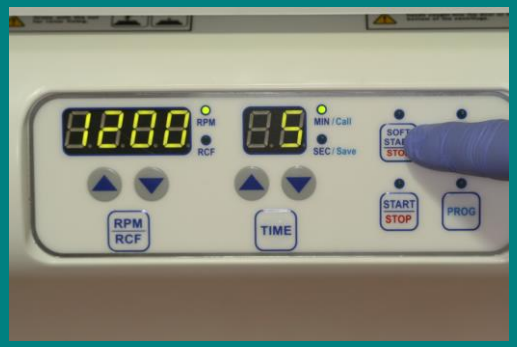


PRP





Centrifugation of the blood



platelet - poor plasma (PPP)

platelet – rich plasma PRP

„the buffy coat"

red blood cells (RBC)

Plasma fractions:

PPP : acellular plasma

PRP : plasma with concentrated number of platelets

contain : fibrinogen
: clotting factors

formation of **fibrin** : will provide the natural *osteoconductive matrix* needed in the **bone regeneration**

Stem cells

Platelets

Macrophages

PRP

Life span of platelets in wound : less than 5 days
(direct influence of GF-s)

Extension of healing : two mechanisms:

1. Increase and activation of **marrow stem cells** into osteoblasts

↓ secrete

TGF-β
IGF
into ***osteoid*** matrix *(Mustoe T.A.)*

2. Chemotaxis and activation of macrophages (replace the platelets as the primary source of growth factors after the third day) *(Pierce G.P.)*;
:PDGF, TGFα, TGFβ1 *(Rappolec D. A.)*

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bone and cementum contain GFs like:
IGF-I,-II, $TGF\beta_1$, PDGF
which may be released following injury *(Hauschka P.V.; Miki Y.)*

PDGF

Platelet – derived growth factor :

- a cationic protein (glycoprotein)
- a polypeptide, stable up to 100 °C

- first found in platelets

/+ macrophages, endothelial cells *(Ross R.)*, monocytes, fibroblasts, bone matrix /

PDGF : is **located** in the *alpha granules* of the platelet
: is **released** from platelets at sites of *vascular damage*

PDGF → circulation → on sites of damage and wounds (proliferative response)

→ restitution of tissue continuity

Roles :

- in process of initiation of *fibroplasia* in wound repair *(Ross R.)*
- stimulates the metabolism and multiplication of connective tissue cells

↓

connective tissue formation in healing wound *(Bowen-Pope D.F.)*

- favors *angiogenesis*:

PDGF → act. → macrophages ↓ secr. factors ↓ endothelial cells → **new capillary sprouts**

- up-regulation of other growth factors and cells

PDGF

as the first growth factor in the wound leads to:

- revascularization
- collagen synthesis
- bone regeneration

PDGF

All isomorphs of PDGF have proliferative effect on **PLF** (PDL fibroblasts) in vitro.

(Matsuda N. , Oates T. W.)

PDGF is chemotactic for **PLF** and promotes collagen and total protein synthesis.

(Matsuda N.)

TGF-β

- first isolated from transformed tissues (sarcomes) *(Burgess A.W.)*
- synthesized by platelets /+ found in macrophages, osteoblasts etc./

TGF-β

: is **located** in the *alpha granules* of the platelet
: is **released** by platelet *degranulation* + actively secreted by *macrophages*

TGF- β : a superfamily of growth factors

- the bone morphogenetic proteins (BMPs) are members *(Celeste A. J.)*
  : the only growth factors known to provoke bone formation heterotopically by making undifferentiated mesenchymal cells differentiated into osteoblasts (osteoinduction) *(Solheim E.)*

TGF-β

Roles

- enlarges the rate of *stem cell proliferation*:

favors → **bone formation**

- can initiate bone regeneration

- can sustain : long – term healing

: bone remodelling of a maturing bone graft

- chemotaxis and mitogenesis of osteoblast precursors

- *inhibition of osteoclast formation* and bone resorption

(Mohan S; Pierce G. F.)

Bio-Oss®

/Manufacturer: Geistlich, Wolhusen, Switzerland/

SCAFFOLD FOR PRP AND GRAFT MATERIAL

- a bovine derived xenograft
- an inorganic mineral bone matrix
- a low crystalline natural apatite

: N B M

anorganic bovine bone mineral **(ABBM)**

Properties:

- **osteoconductive**

(Hämmerle, 1998; Valentini, Maiorana, 2000)

- **chemical and physical properties are identical to human bone**

(Giovanoli, 1994; Valdre, 1995)

- **role in the regenerative processes**

(Hürzeler, 1997; Mellonig, 2000)

Cerasorb®

/Manufacturer: Curasan Pharma, Kleinostheim, Germany/

- a beta-tricalciumphosphate (**ßTCP**)

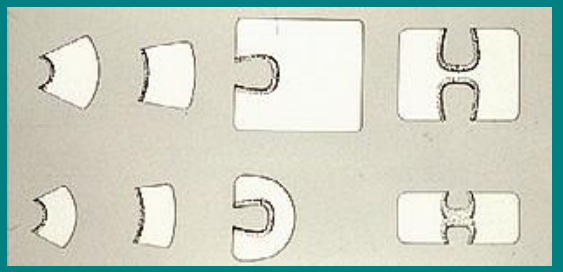
Properties:

- **osteoconductive**
- **micromorphology: interconnecting pores**
- **resorbability**
- **biocompatibility**
- **independent of pH changes**
- **synthetic, inorganic**

GTR

ePTFE Gore - Tex® Membrane

/Manufacturer: W. L. Gore & Associates Inc. Flagstaff, Arizona, USA /



- expanded polytetrafluoroethylene

- a passive non-resorbable membrane

*

Bio-Gide® Perio Membrane

/Manufacturer: Geistlich, Wolhusen, Schweiz/

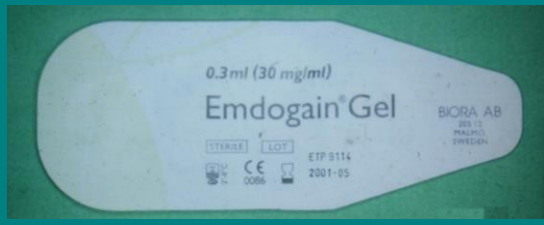
- resorbable double-layered – collagen membrane

EMD

Emdogain®

/Manufacturer: Straumann Biologics, Schweiz/

- enamel matrix proteins



Curasan PRP Kit

PRP

/Curasan Pharma GmbH, Kleinostheim,Germany/

-8.5 ml of citrated blood (CPDA citrate phosphate dextrose adenine) were centrifuged in a standard laboratory centrifuge (Hettich EBA 8S) for 10 minutes at 2400 rpm (1.)

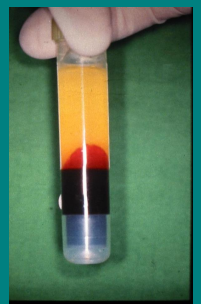
-the yellow plasma (containing the *thrombocytes*) was taken up together with the upper 3 mm layer of RBCs (containing *fresh thrombocytes* too) into a monovette with a long cannula, using an additional air - intake cannula

-second centrifugation was performed for 15 minutes at 3600 rpm (2.)

-the thrombocyte pellet was *resuspended* in the residual 0.3 - 0.4 ml plasma using a conventional shaker (Scientific Industries Vortex-Genie 2)

curasan

1.



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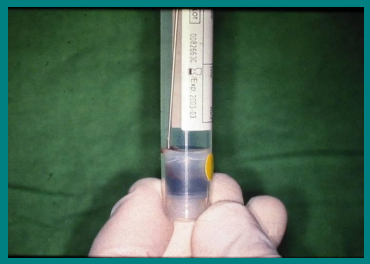
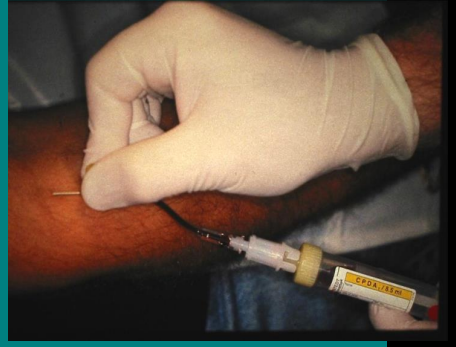
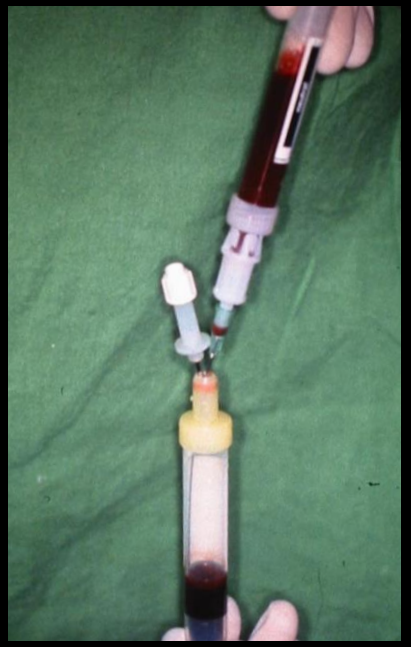


the „pellet" + rest-plasma

PRP

PRP

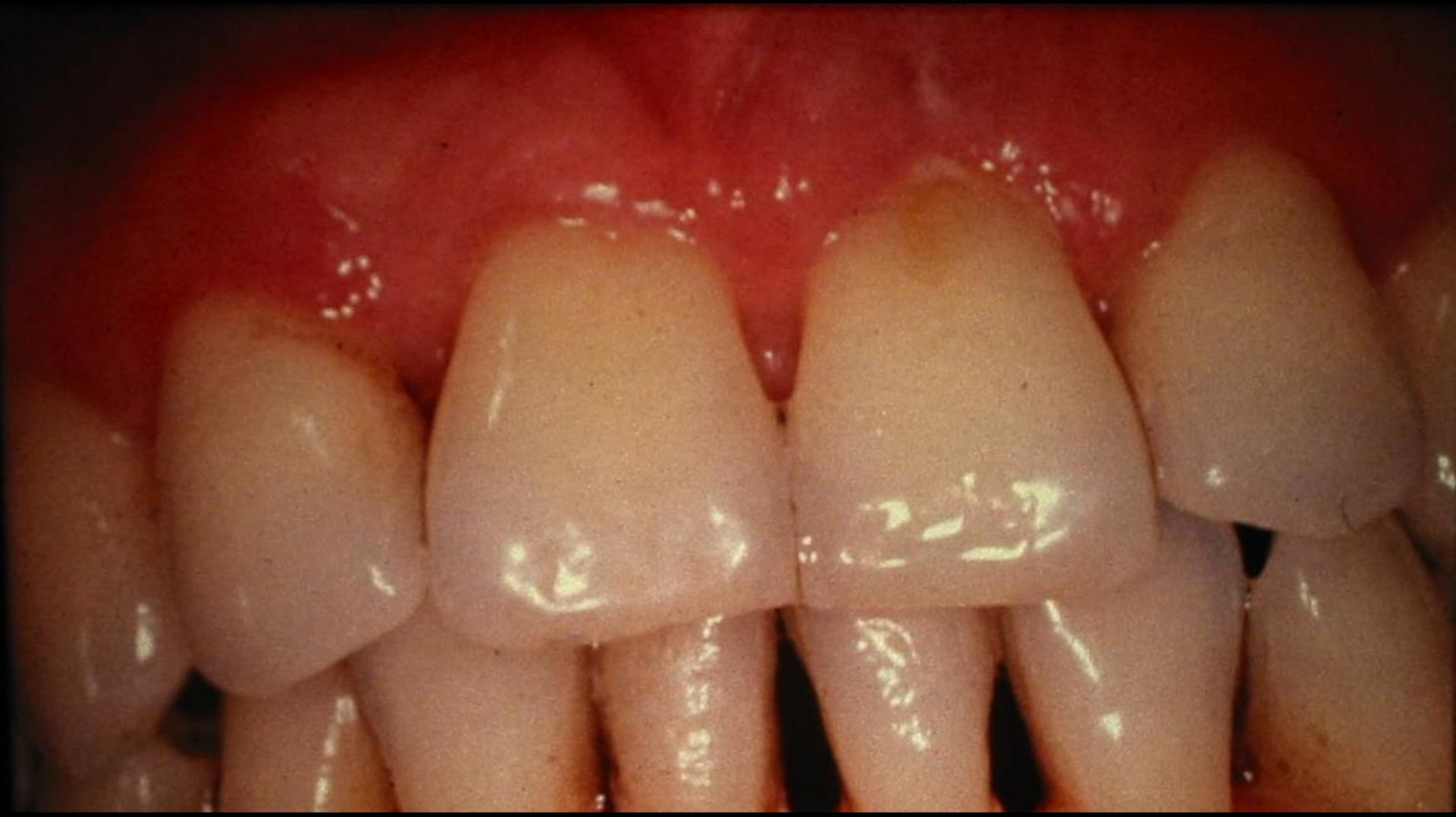




The first 1-3 mm of the **RBC** layer contains the larger and more recently synthesized platelets - this layer is included in the **PRP**

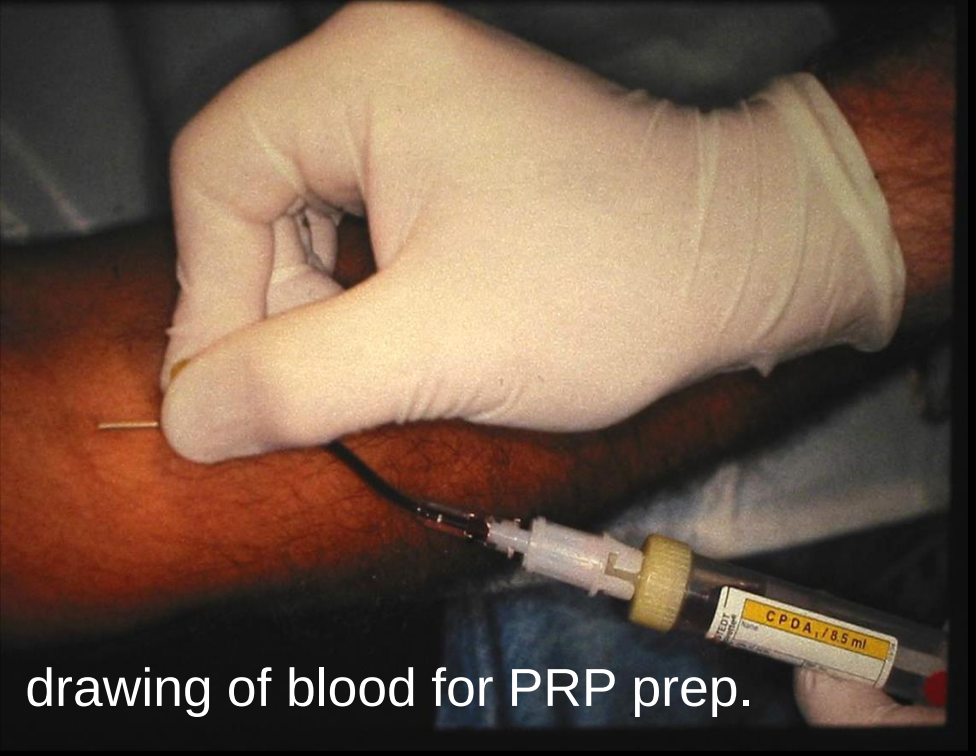
(this will import a *red tinct* to the otherwise straw – colored **PRP**)

(Marx R. E.)

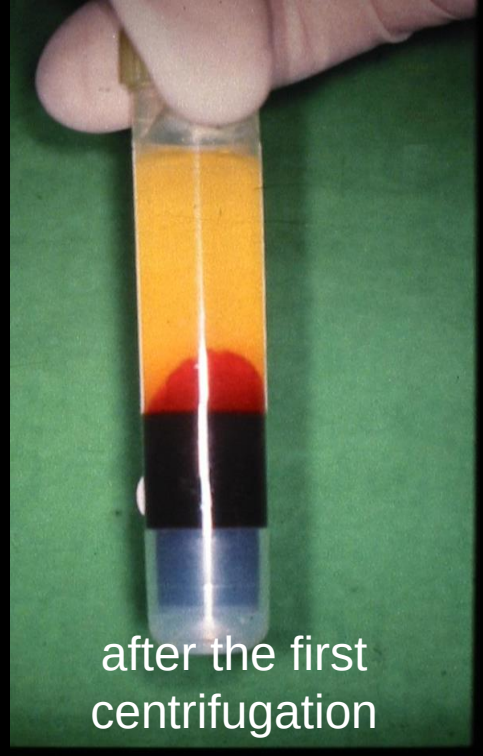


preop.

PRP+NBM+GTR



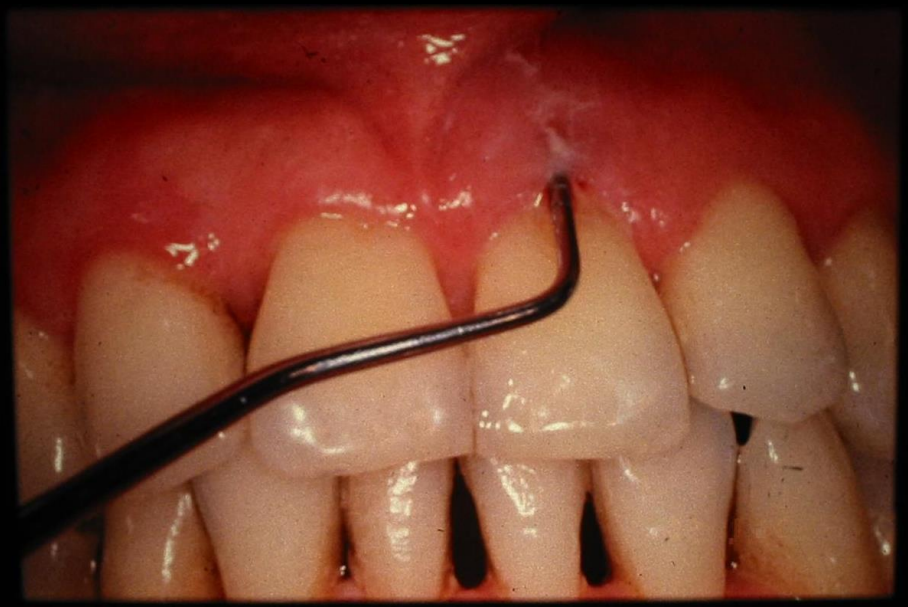
drawing of blood for PRP prep.

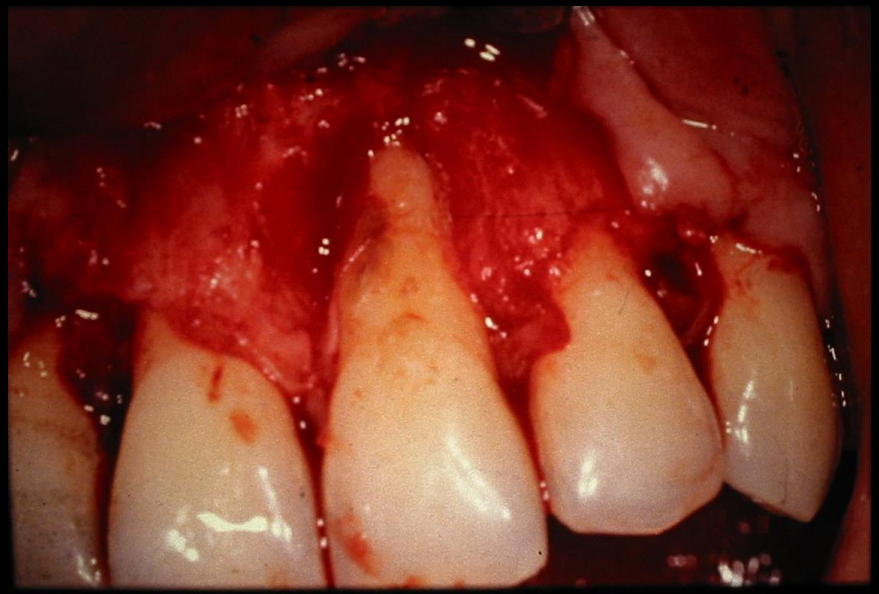

after the first centrifugation

after the second centrifugation

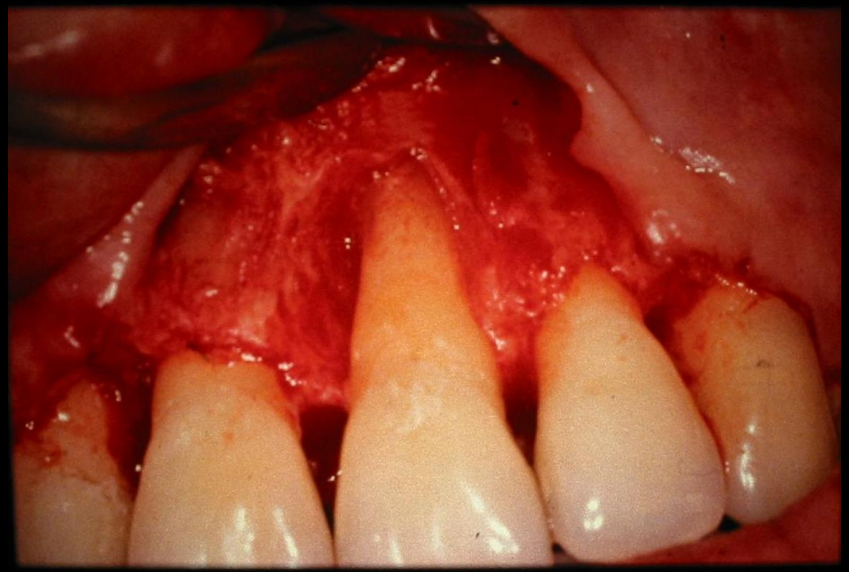
pellet
+
rest plasma

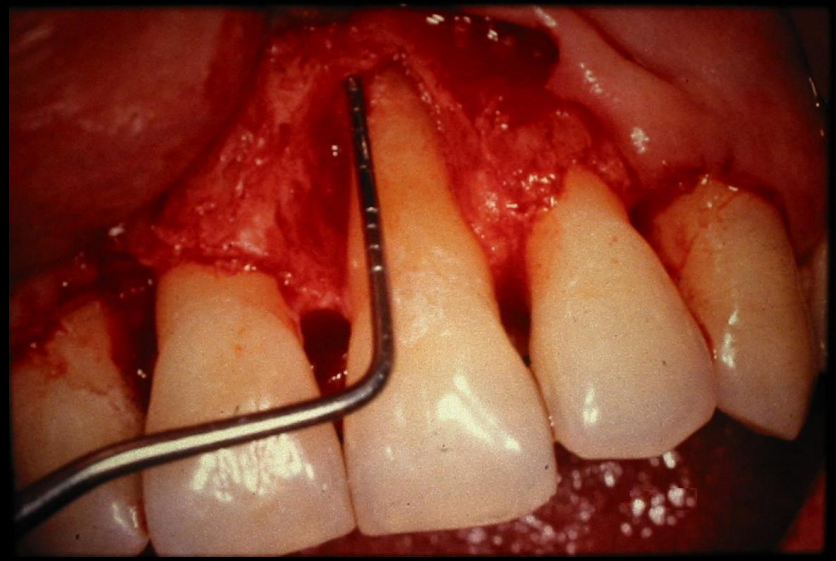

adding PRP to NBM



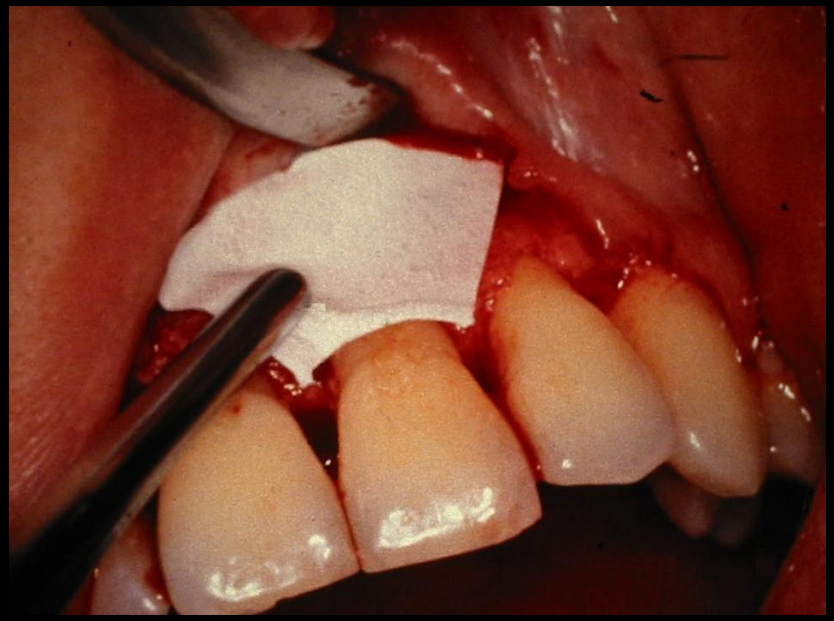


preop.

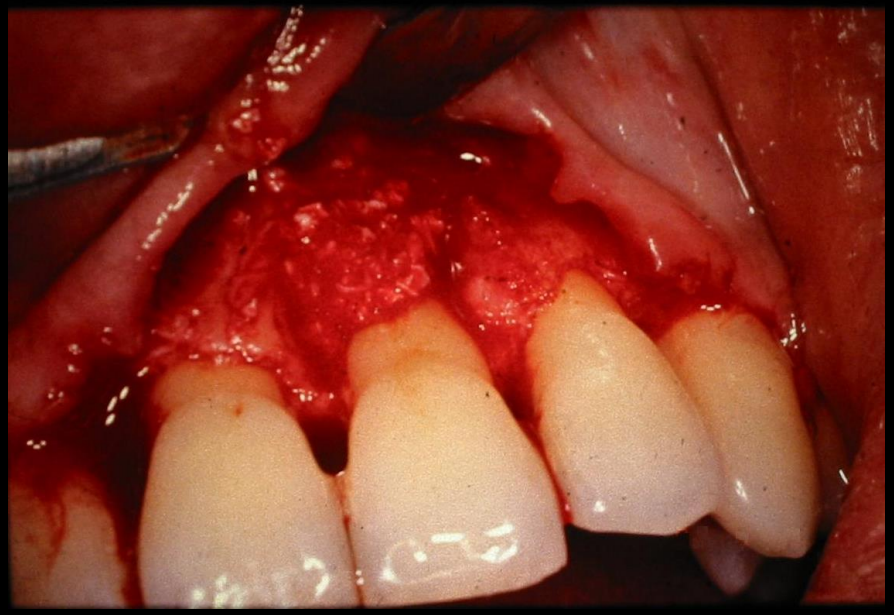




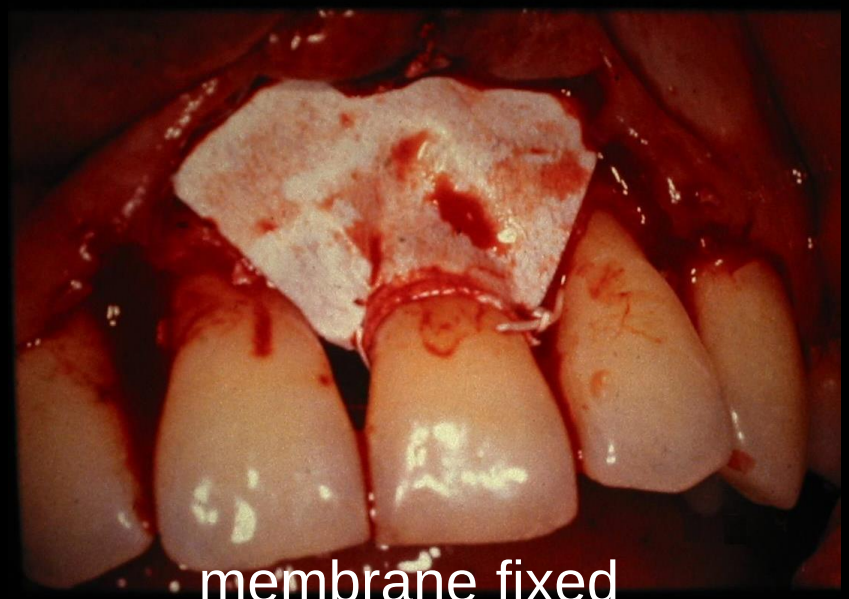
the defect



checking the membrane

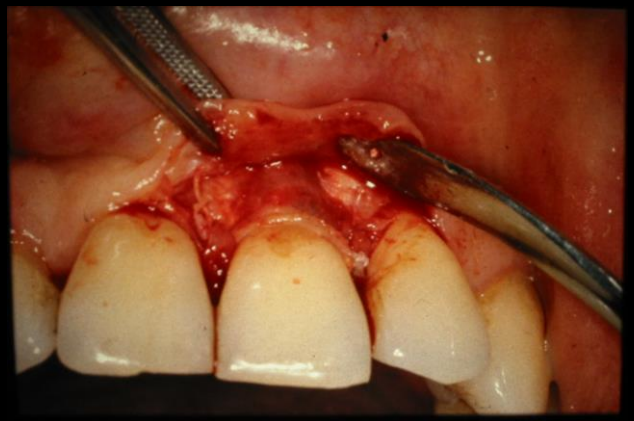


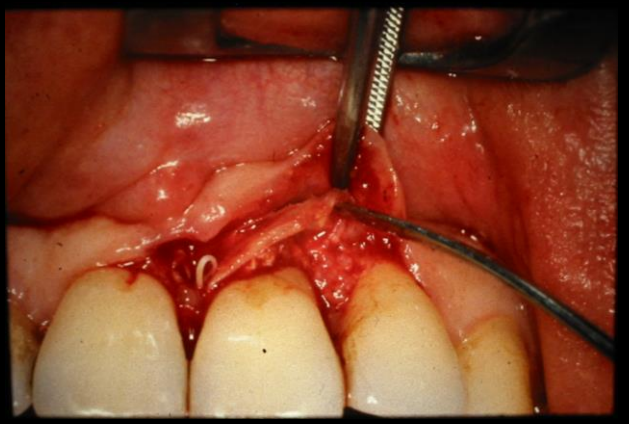
NBM + PRP placed in defect



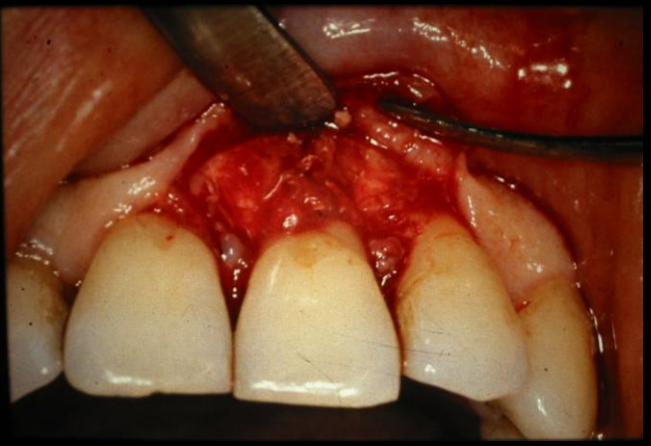
membrane fixed
over implanted material

sutures

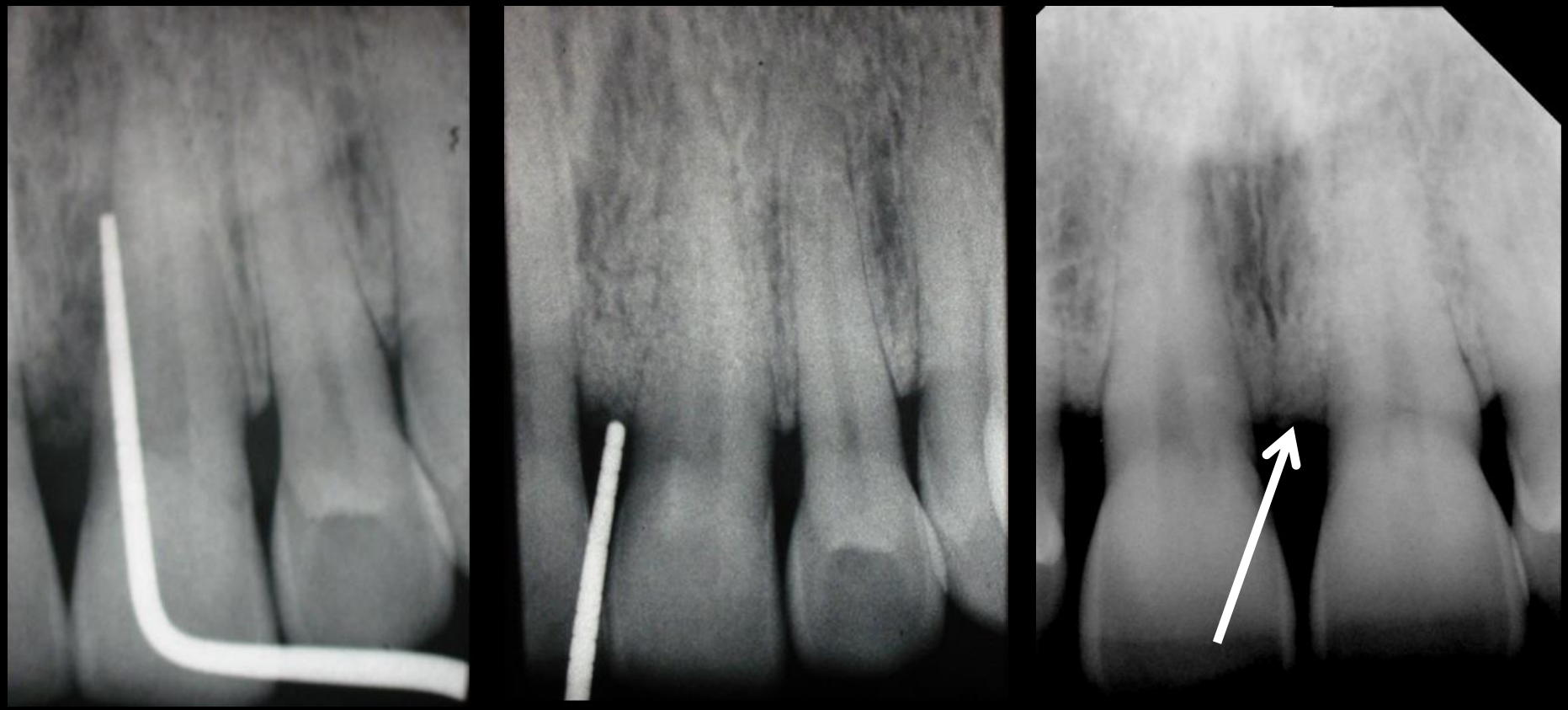




membrane removal after 6 weeks

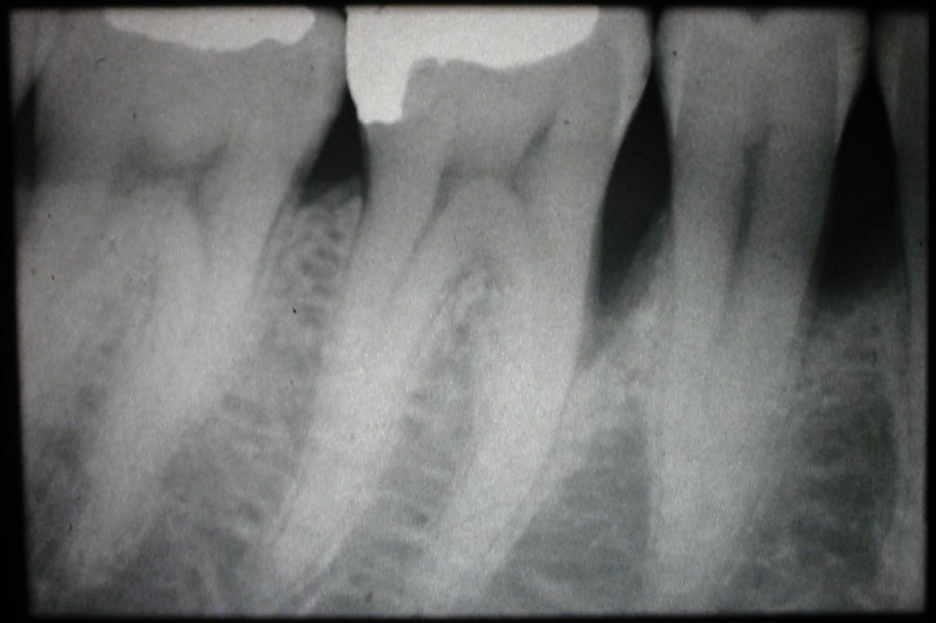


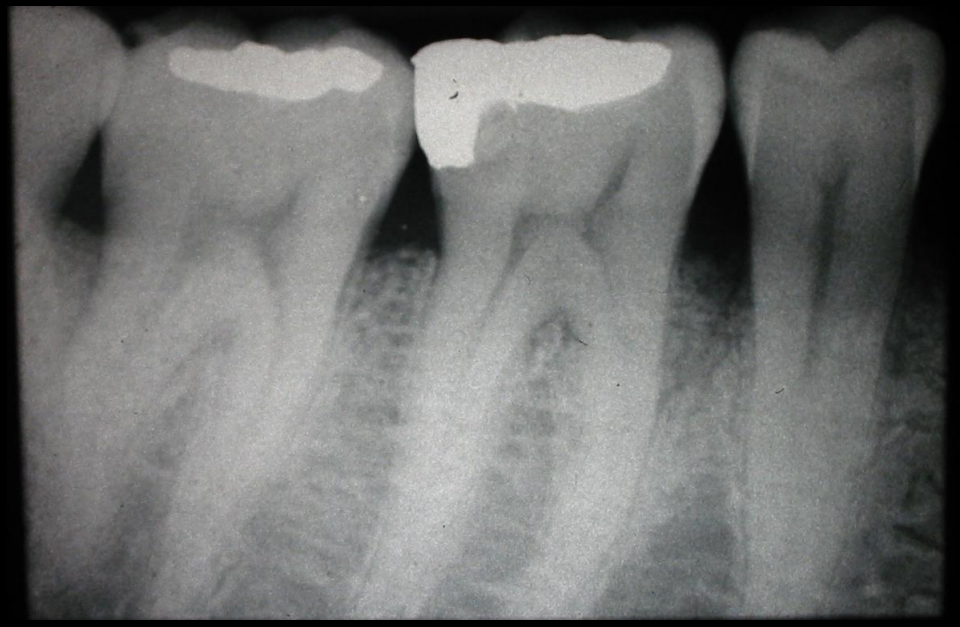
postop. after 6 months



preop. after 1 year after 8 years

PRP + NBM + GTR

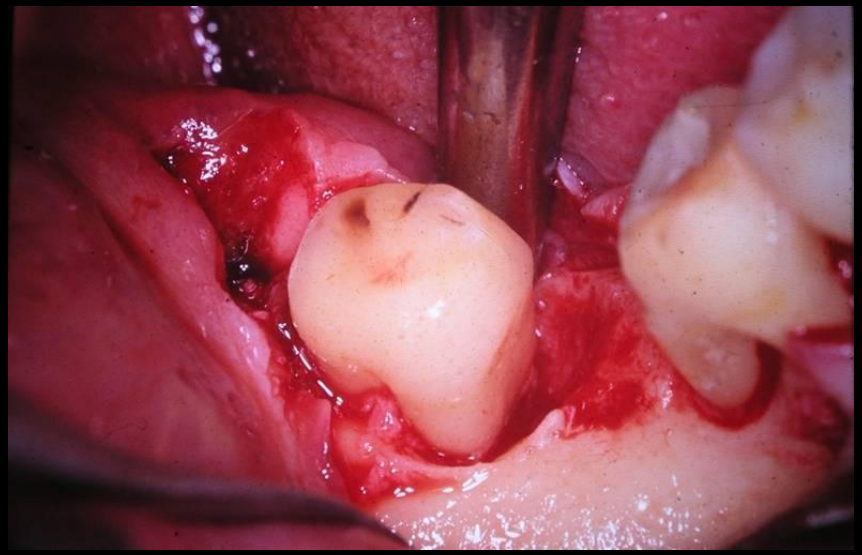




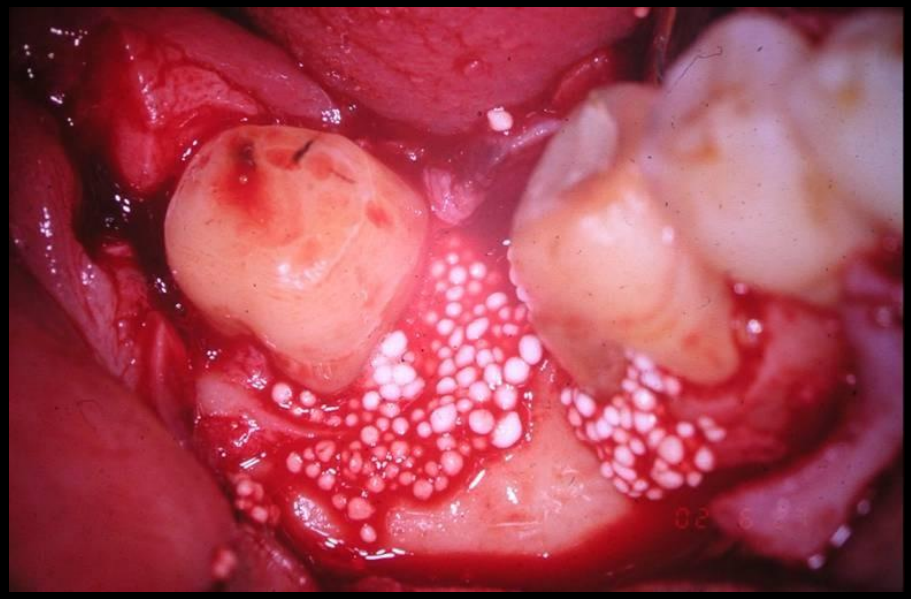
preop.

after 12 months

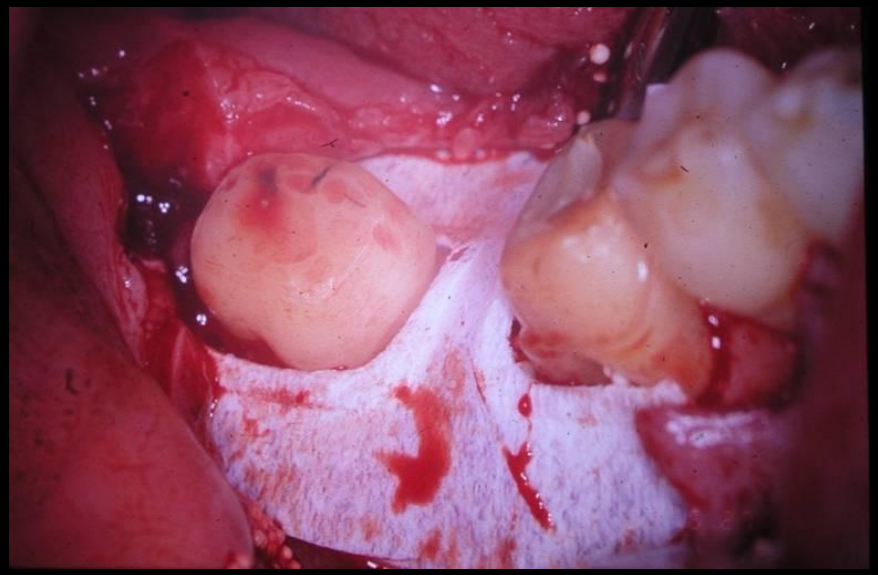
preop.



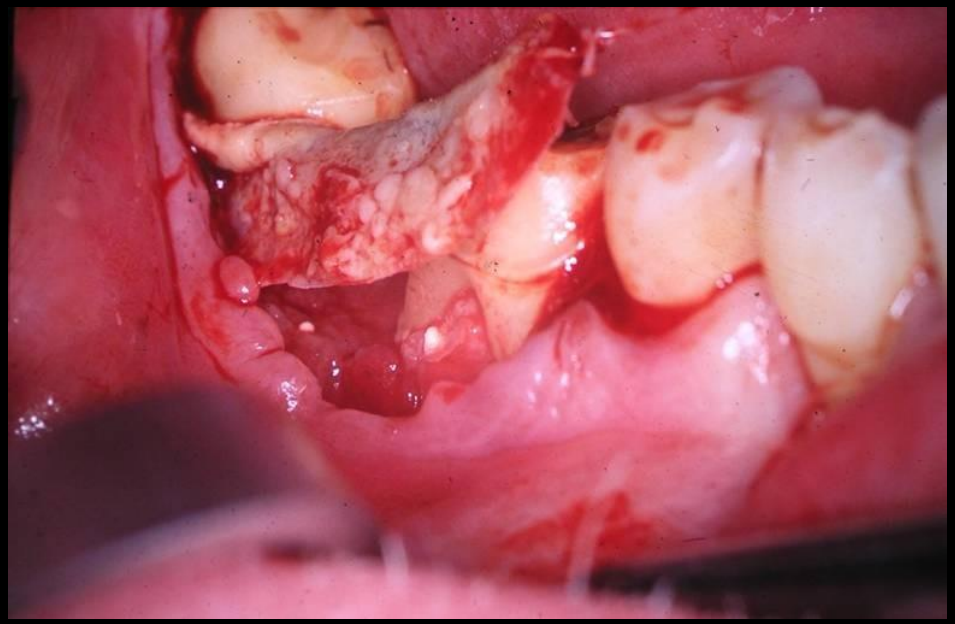
intrabony defect



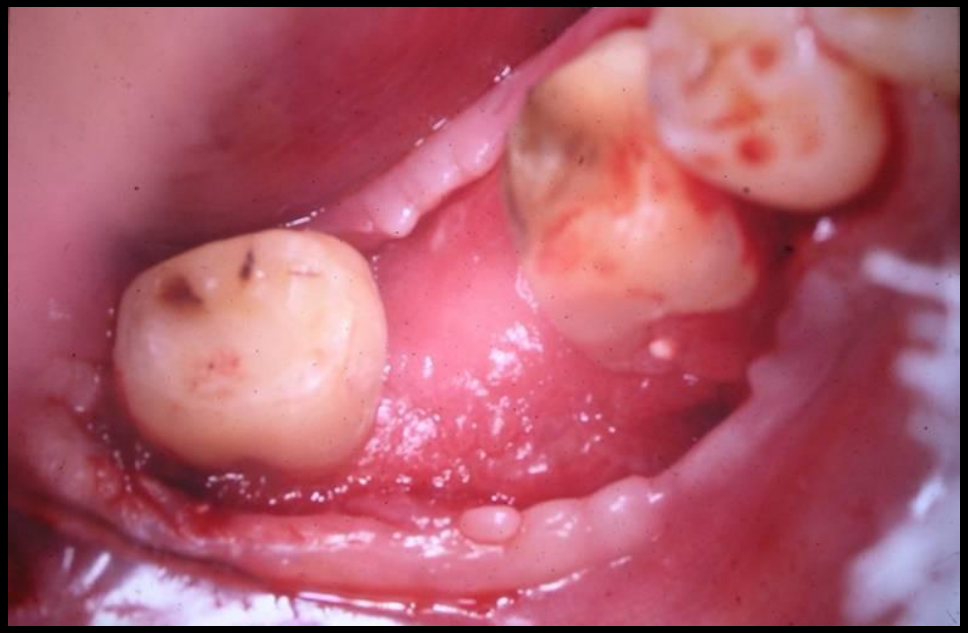
PRP + β-TCP in defect



coverage with e-PTFE barrier

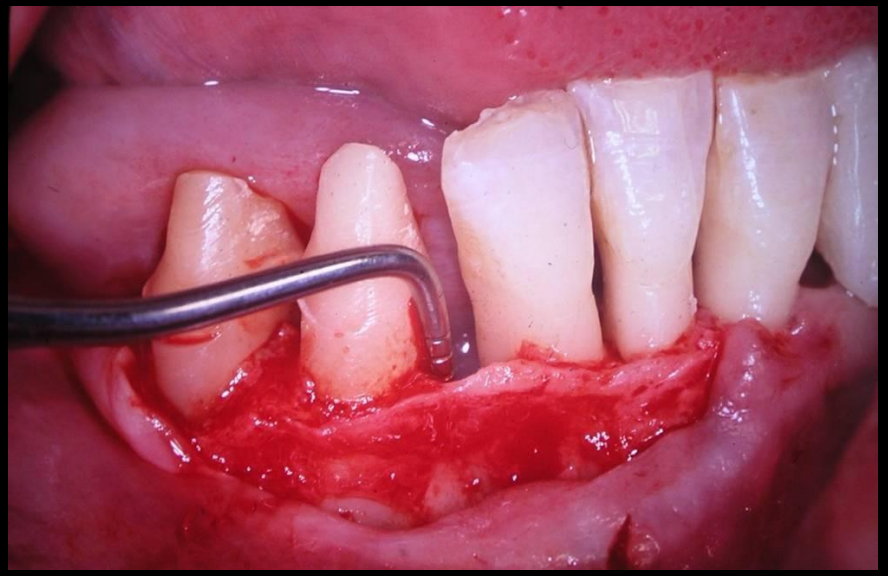


membrane removal
after 6 weeks

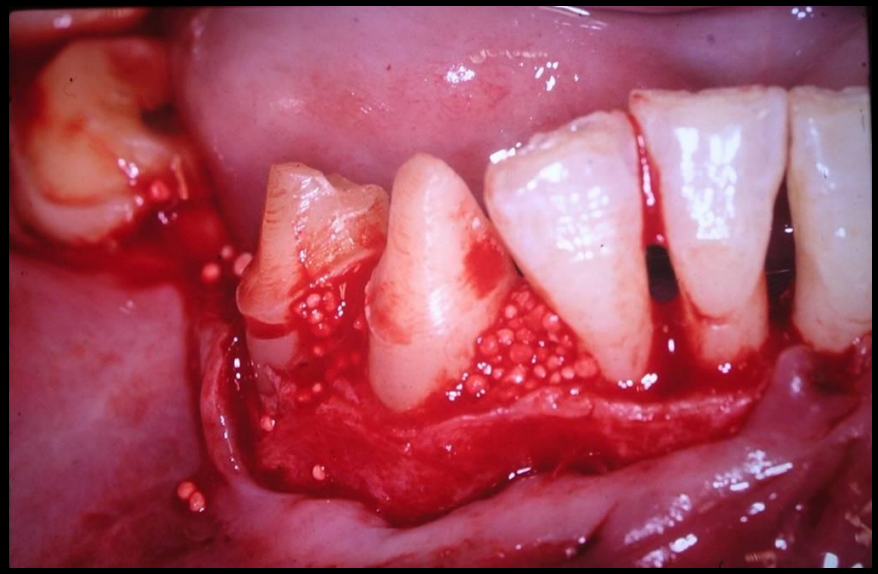


„re-entry” after 6 weeks

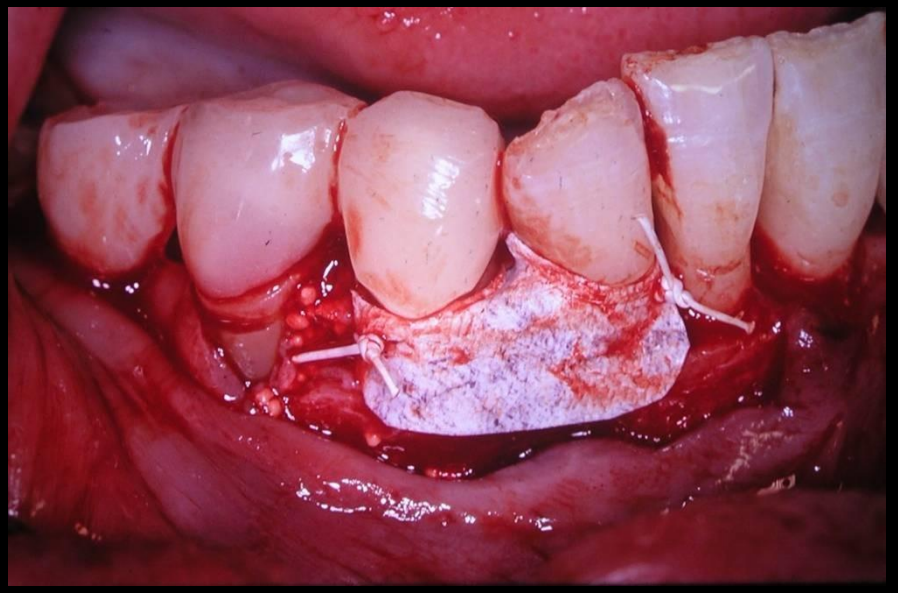
preop.



the mesiolingual defect



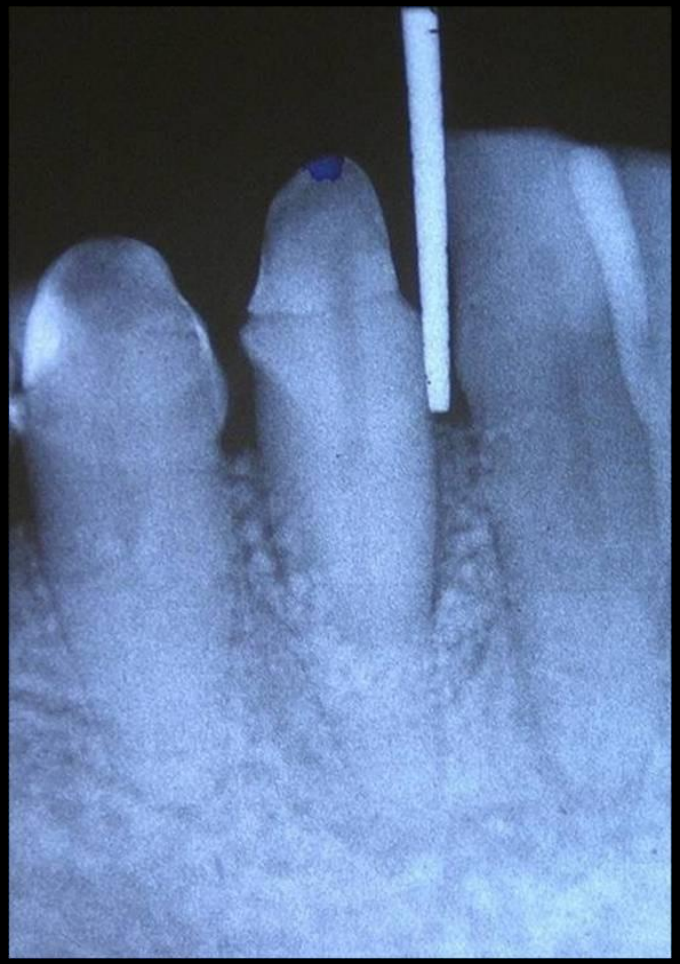
PRP + β-TCP



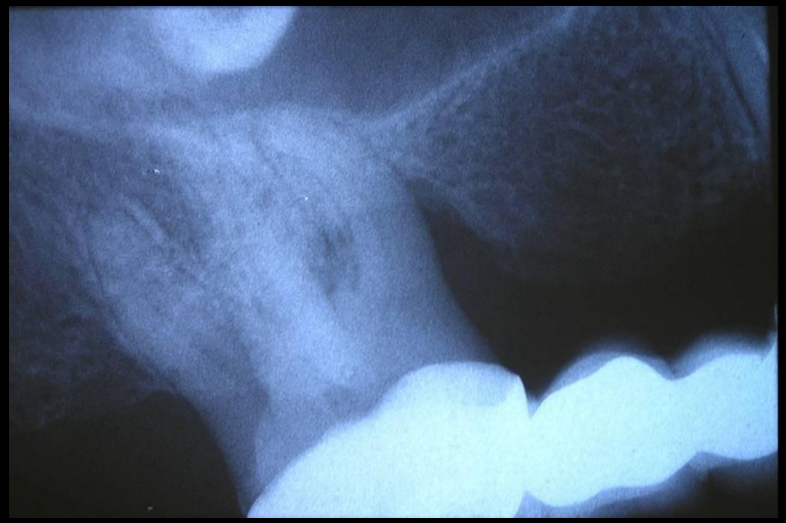
coverage with e-PTFE barrier

PRP + β-TCP + GTR

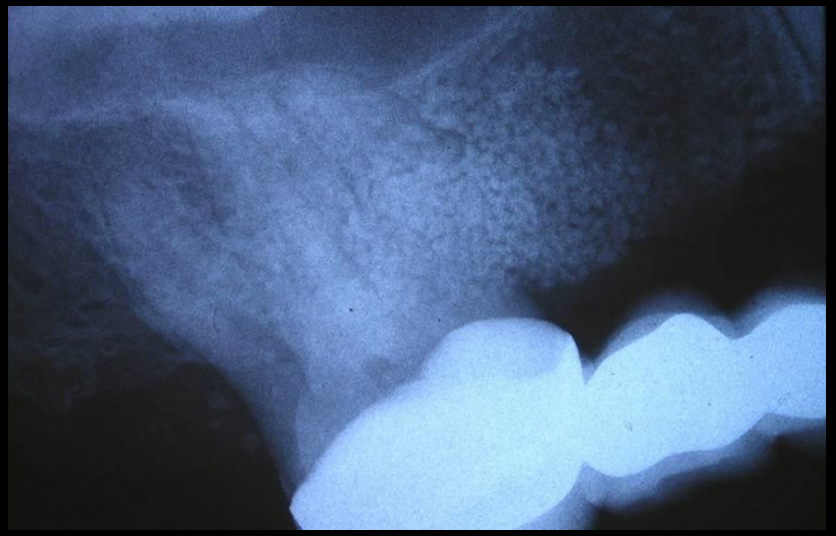
preop. X-ray picture



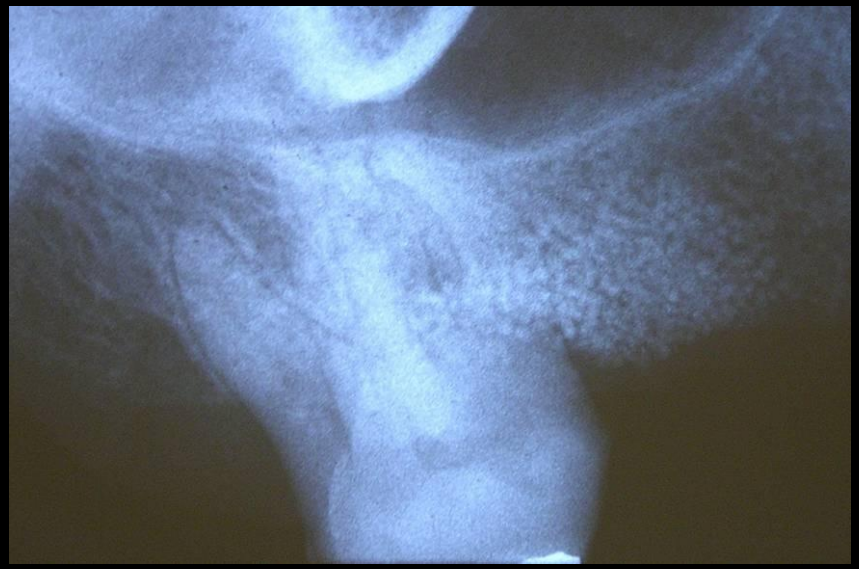
X-ray control after 6 months



preop. X-ray picture



X-ray picture after surgery

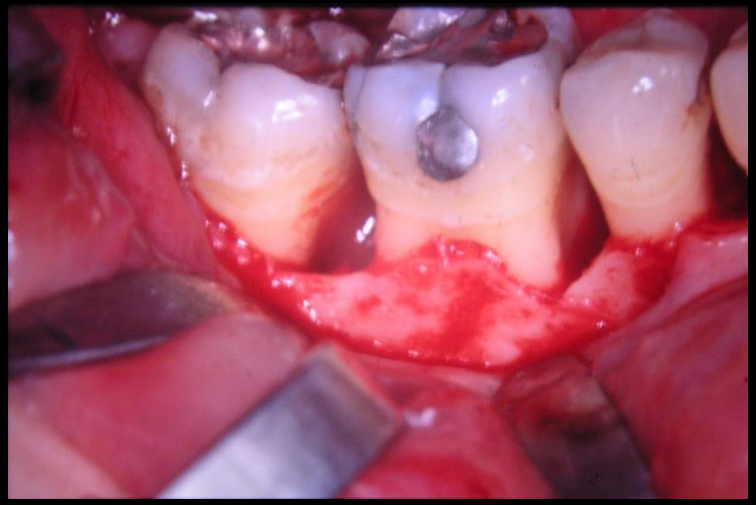


X-ray control after 12 months

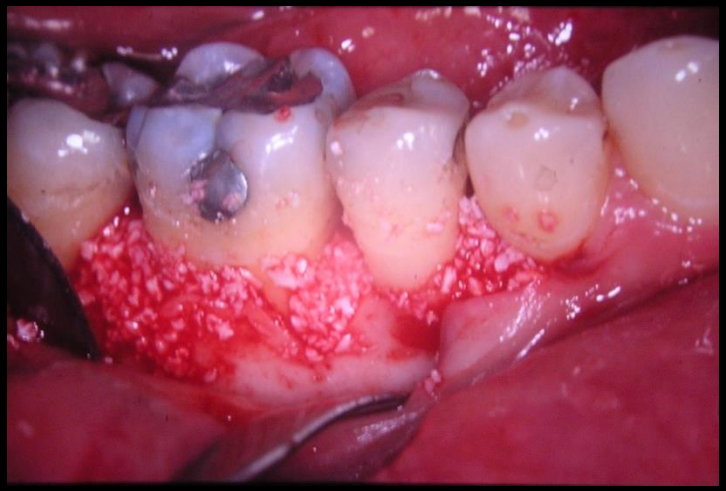
PRP + β-TCP + GTR

PRP + NBM + GTRr

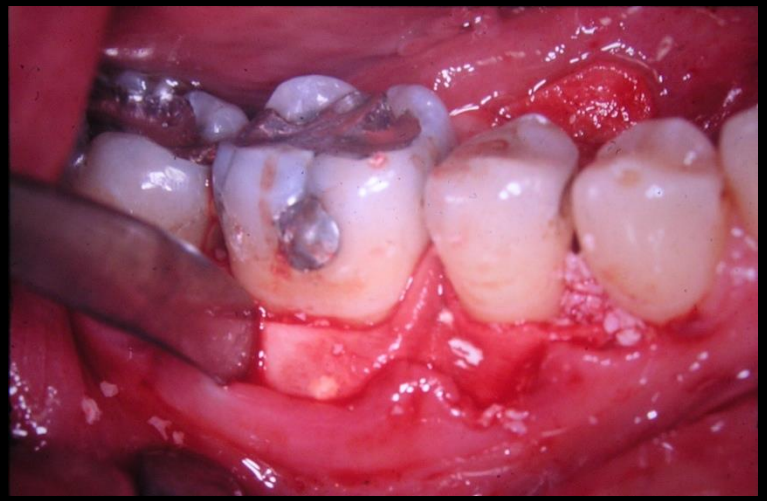
preop. clinical picture



intrabony defects



Bio-Oss + PRP in defects



coverage with the collagen membrane (Bio-Gide Perio)

PRP + NBM + GTRr

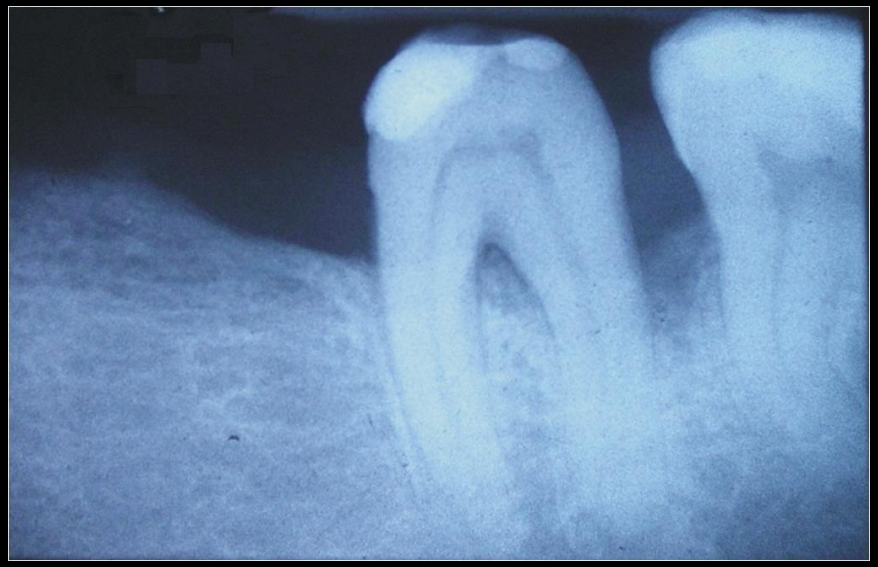
preop.

postop. one week

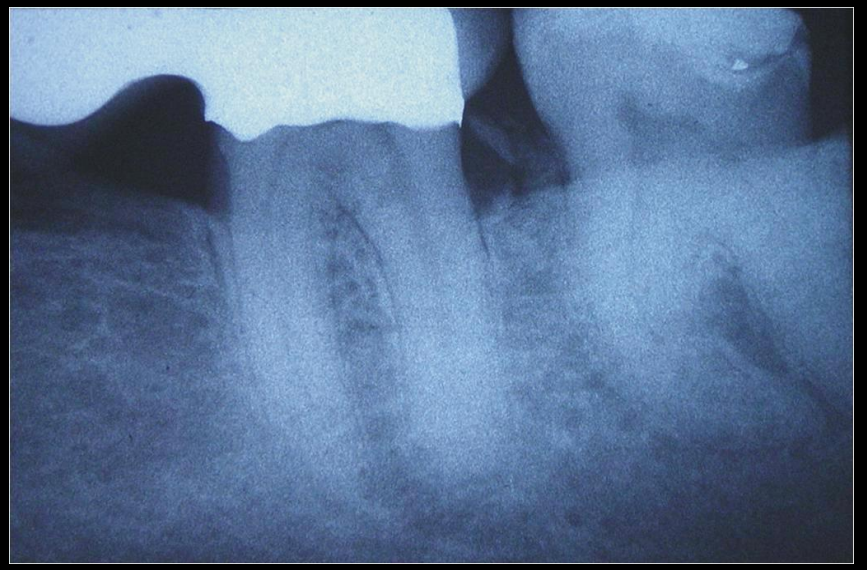
postop. one year

clinical control
one year postop.

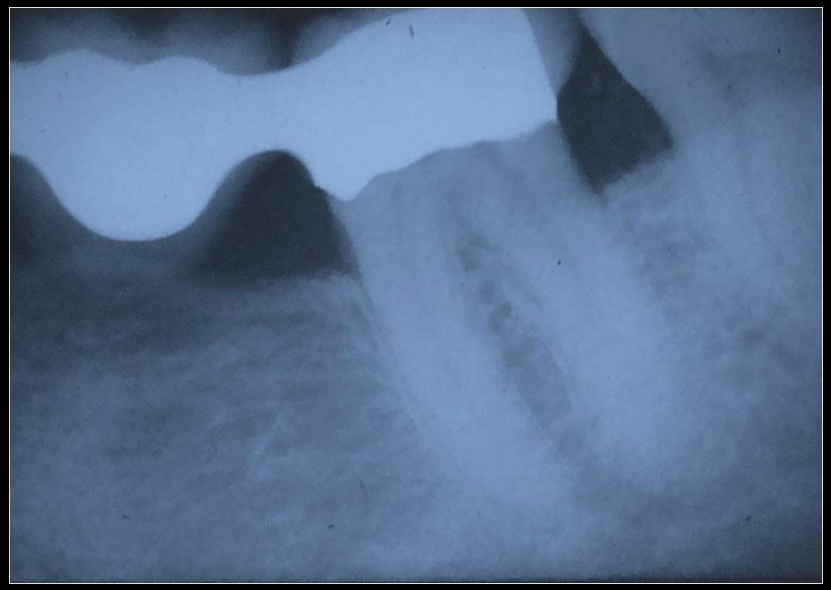
PRP + NBM



preop.



after 1 year



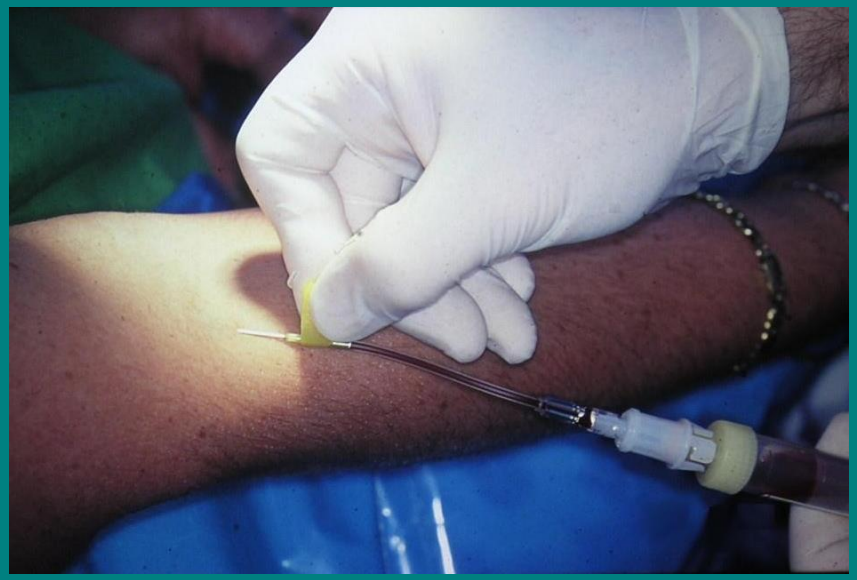
after 5 years

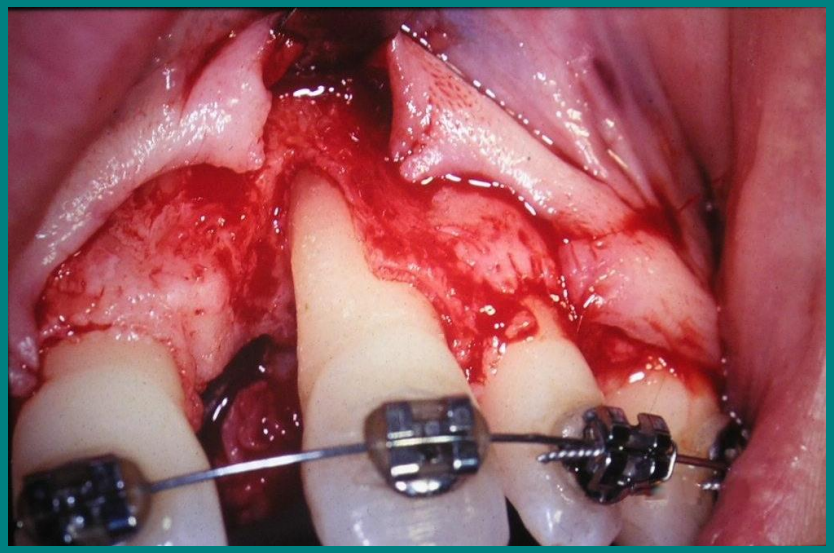
PRP + NBM

preop.

after 1 year

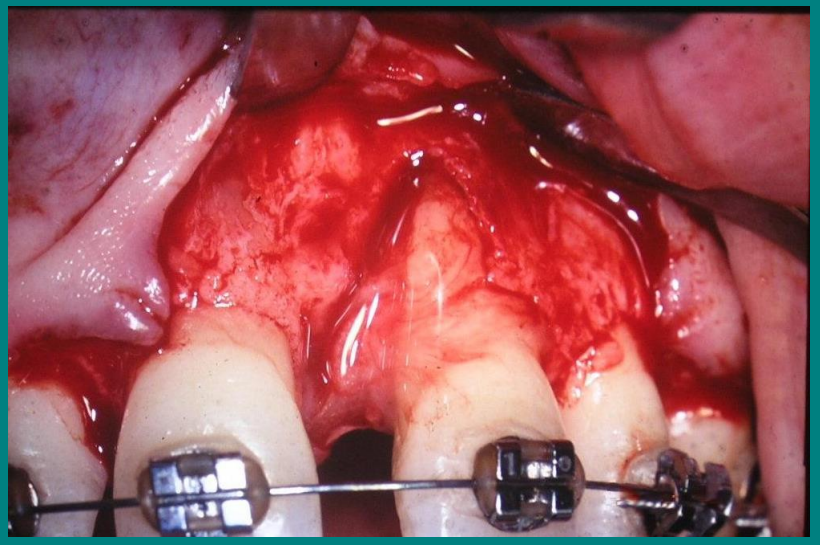
after 5 years



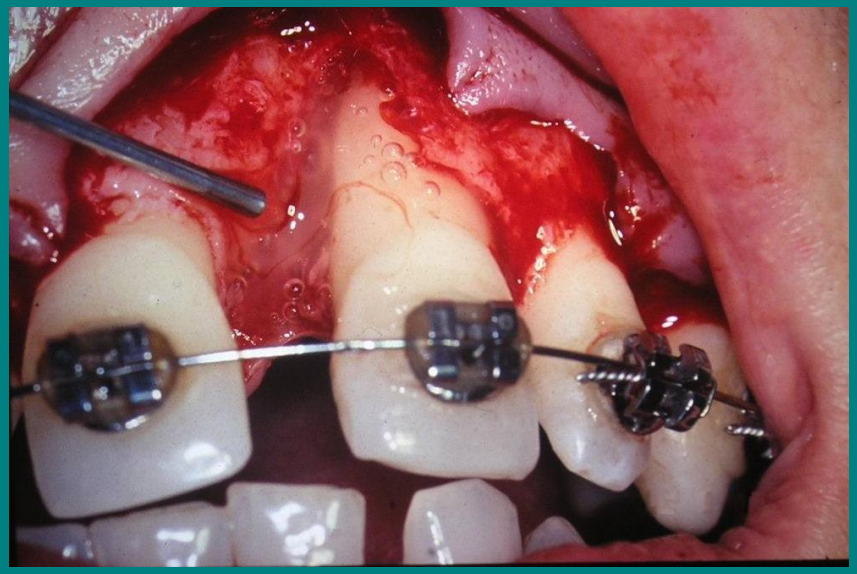


the defect

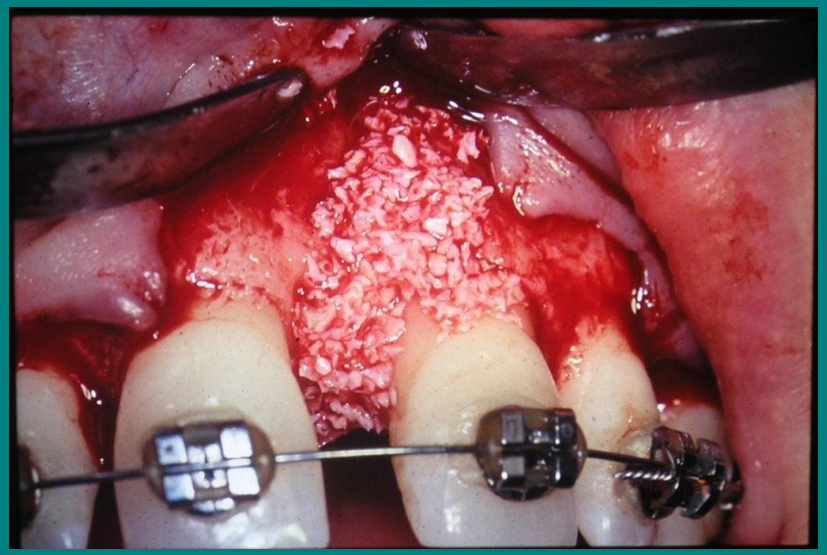
NBM + PRP + EMD



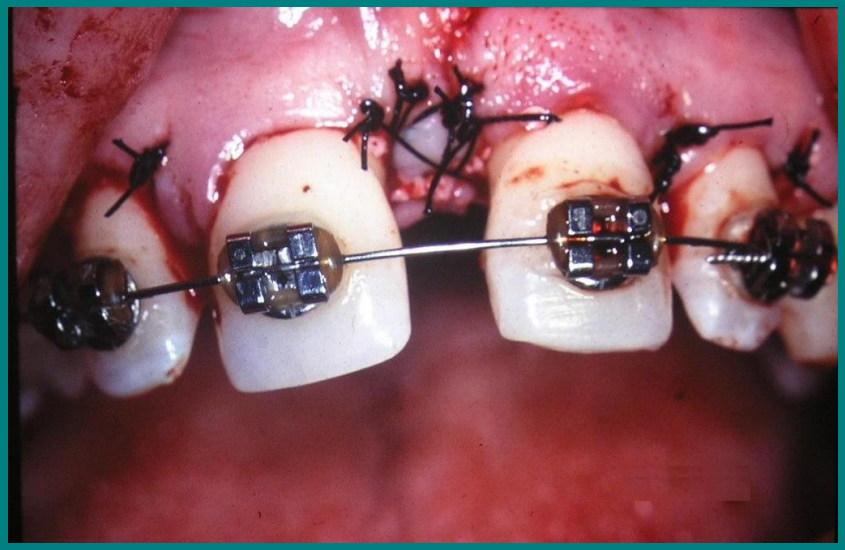
conditioning with EDTA



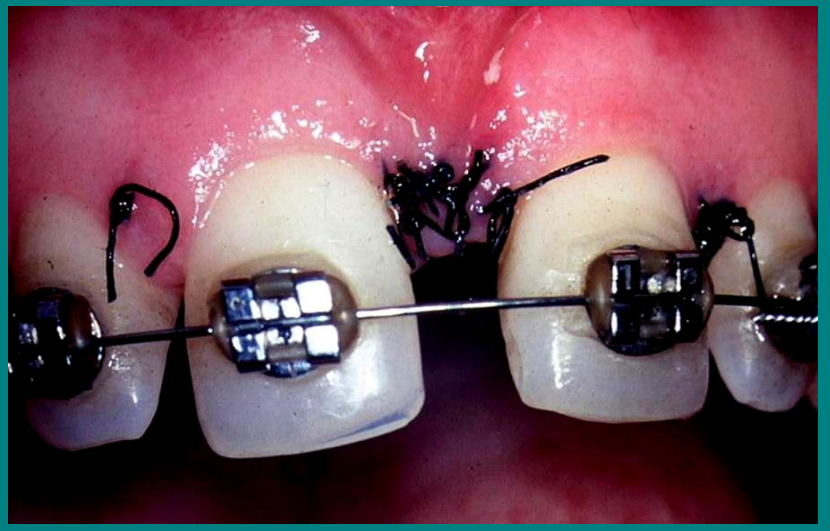
application of Emdogain



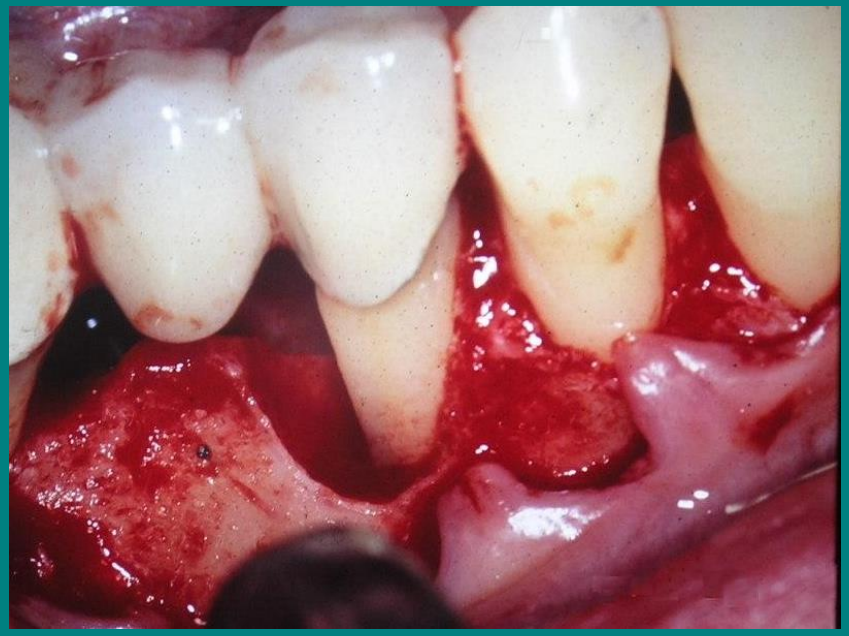
NBM + PRP in the defect



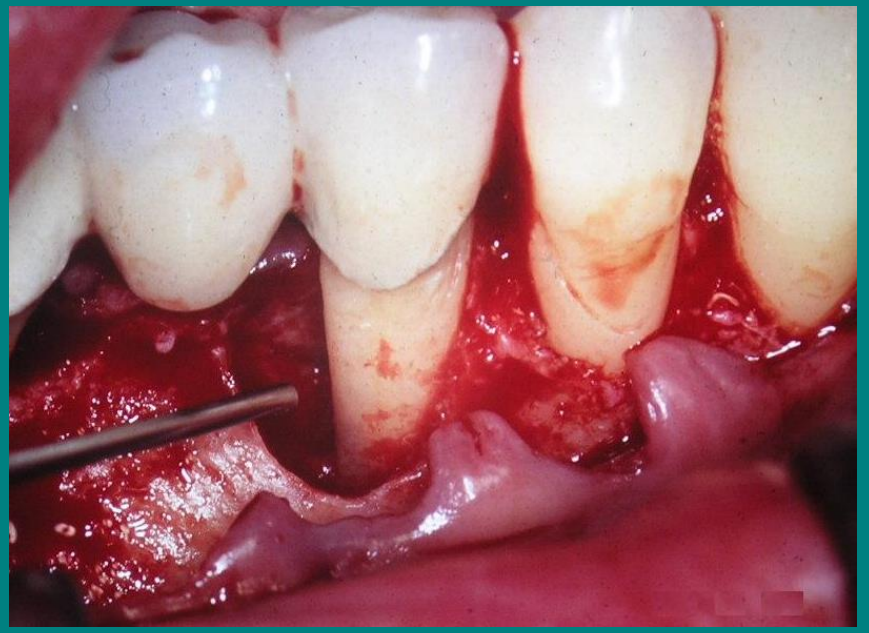
sutures



10 days after surgery



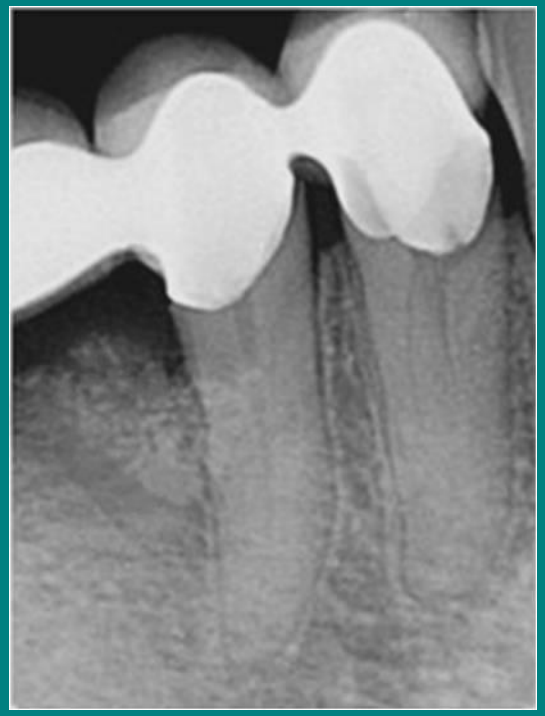
deep intrabony defect



EMD

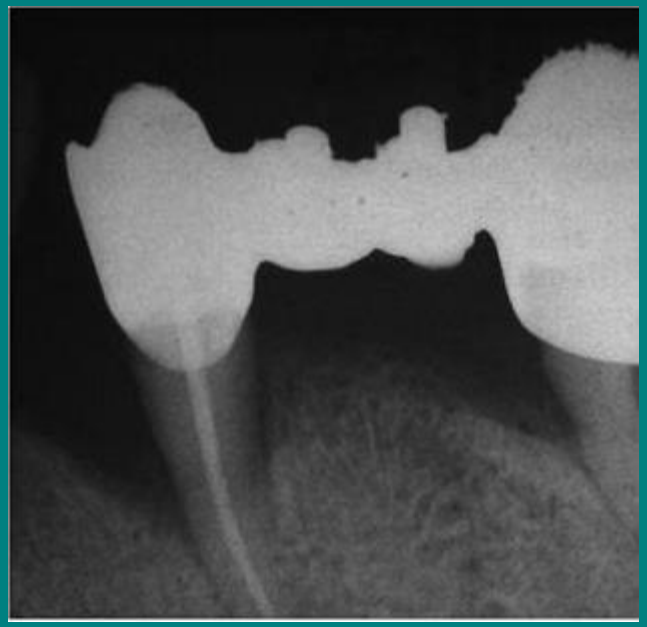
NBM + PRP

sutures

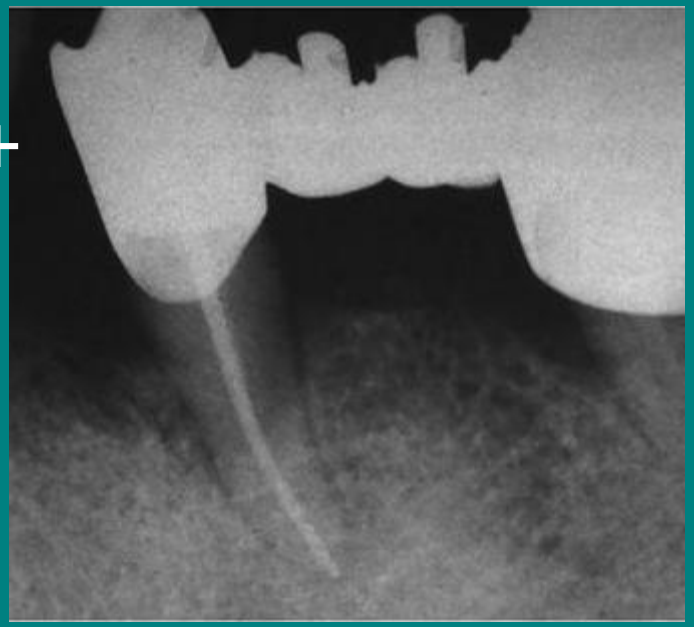


preop. 1 year 5 years

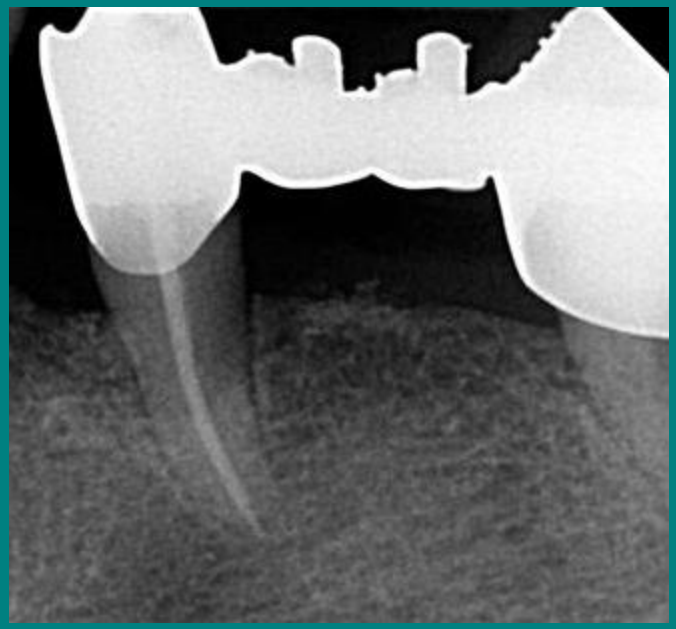
NBM + PRP + EMD



NBM + PRP + EMD



preop.



1 year

5 years

Animal studies with PRP

- Positive results

Effect of combining platelet-rich plasma with anorganic bovine bone on vertical bone regeneration: early healing assessment in rabbit calvariae.

Torres J, Tamimi F, Tresguerres IF, Alkhraisat MH, Khraisat A, Blanco L, Lopez-Cabarcos E.

Int J Oral Maxillofac Implants. 2010 Jan-Feb;25(1):123-9.

- Aim: to evaluate the combination of NBM (ABB) with PRP when used in vertical bone augmentation.
- Conclusion: after six weeks the mixture of PRP and NBM produced twice the vertical bone volume of NBM alone.

Evaluation of platelet-rich plasma in combination with anorganic bovine bone in the rabbit cranium: a pilot study.

Aghaloo TL, Moy PK, Freymiller EG.

Int J Oral Maxillofac Implants. 2004 Jan-Feb;19(1):59-65.

- Aim: to compare bone formation in cranial defects grafted with autogenous bone, xenograft, and xenograft with PRP (with a no-graft group as a control).
- Conclusion: the study showed an increase in bone formation with the addition of PRP to Bio-Oss in non-critical-sized defects.

Animal studies with PRP

- Negative results

Effect of solely applied platelet-rich plasma on osseous regeneration compared to Bio-Oss: a morphometric and densitometric study on rabbit calvaria.

Torres J , Tamimi FM, Tresguerres IF, Alkhraisat MH, Khraisat A, Lopez-Cabarcos E, Blanco L.

Clin Implant Dent Relat Res. 2008 May;10(2):106-12.

- Aim: to evaluate the benefits of using PRP alone, and compare it to Bio-Oss in vertical bone augmentation.
- Conclusion: no beneficial effect of using PRP on osseous regeneration. The Bio-Oss presents good osteoconductive properties by achieving suitable bone volume values.

The effect of platelet-rich plasma on early and late bone healing using a mixture of particulate autogenous cancellous bone and Bio-Oss: an experimental study in goats.

Mooren RE , Dankers AC, Merkx MA, Bronkhorst EM, Jansen JA, Stoelinga PJ.

Int J Oral Maxillofac Surg. 2010 Apr;39(4):371-8. Epub 2010 Feb 2.

- Aim: to study the effect of PRP on a mixture of autogenous bone and Bio-Oss particles in goats.
- Conclusion: results of the histological and histomorphometric examination showed that early and late bone healing were not enhanced when PRP was used.

Human studies with PRP

- Positive results

Effect of platelet-rich plasma in the treatment of periodontal intrabony defects in humans.

Ouyang XY, Qiao J.

Chin Med J (Engl). 2006 Sep 20;119(18):1511-21.

- Aim: to evaluate the effectiveness of PRP as an adjunct to NBM (BPBM) graft in the treatment of human intrabony defects.
- Conclusion: treatment with PRP and NBM led to a significantly favorable clinical improvement in periodontal intrabony defects compared to using NBM alone.

Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial.

Hanna R , Trejo PM, Weltman RL.

J Periodontol. 2004 Dec;75(12):1668-77.

- Aim: to compare the clinical outcomes obtained by the combination of PRP and NBM (BDX) to those obtained from the use of the graft alone.
- Conclusion: the addition of PRP to the xenograft significantly improved the clinical periodontal response after 6 months.

Human studies with PRP

- Negative results

A surgical reentry study on the influence of platelet-rich plasma in enhancing the regenerative effects of bovine porous bone mineral and guided tissue regeneration in the treatment of intrabony defects in humans.

Camargo PM , Lekovic V, Weinlaender M, Divnic-Resnik T, Pavlovic M, Kenney EB.

J Periodontol. 2009 Jun;80(6):915-23.

- Aim: to evaluate the additional benefits provided by the incorporation of PRP into a regenerative protocol consisting of NBM (BPBM) and GTR in the treatment of intrabony defects.
- Conclusion: PRP did not significantly augment the effects of NBM and GTR.

An evaluation of platelet-rich plasma without thrombin activation with or without anorganic bone mineral in the treatment of human periodontal intrabony defects

Rodrigues SV, Acharya AB, Thakur SL.

Platelets. 2011 Mar 7. [Epub ahead of print]

- Aim: to evaluate the efficacy of PRP without thrombin activation, alone or in combination with NBM (ABM).
- Conclusion both PRP and PRP combined with NBM results in significant clinical improvement. Statistically insignificant, there are better clinical results with the addition of NBM to PRP.

Human studies with PRP

- Negative results

Effect of platelet-rich plasma on the healing of intra-bony defects treated with a natural bone mineral and a collagen membrane.

Dőri F, Huszár T, Nikolidakis D, Arweiler NB, Gera I, Sculean A.
J Clin Periodontol. 2007 Mar;34(3):254-61. Epub 2007 Jan 25.

- Aim: to clinically compare treatment of deep intra-bony defects with NBM+PRP+GTR or NBM+GTR.
- Conclusions: at 1 year after surgery, significant PD reductions and CAL gains were found in both groups, but the use of PRP has failed to improve the results.

Effect of platelet-rich plasma on the healing of intrabony defects treated with Beta tricalcium phosphate and expanded polytetrafluoroethylene membranes.

Dőri F, Huszár T, Nikolidakis D, Tihanyi D, Horváth A, Arweiler NB, Gera I, Sculean A.
J Periodontol. 2008 Apr;79(4):660-9.
Aim: to clinically evaluate the effect of PRP on the healing of deep intrabony defects.
Conclusion:: significant PD reductions and CAL gains were observed. No statistically significant differences were observed between the two groups at the 1-year reevaluation.

Effect of platelet-rich plasma on the healing of intrabony defects treated with an anorganic bovine bone mineral: a pilot study.

Dőri F , Kovács V, Arweiler NB, Huszár T, Gera I, Nikolidakis D, Sculean A.
J Periodontol. 2009 Oct;80(10):1599-605.

- Aim: to clinically compare the healing of intrabony defects treated with either a combination of NBM (ABBM) and PRP to those obtained with NBM alone.
- Conclusion: at 1 year after surgery, significant PD reductions and CAL gains were found, and the use of PRP failed to improve the results obtained with NBM alone.

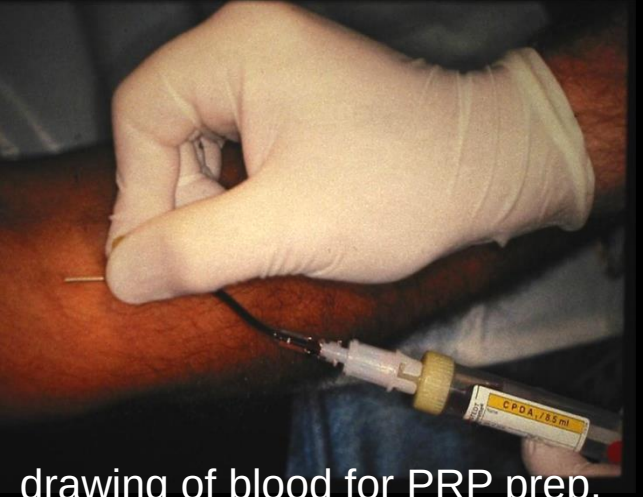
Studies with PRP (review)

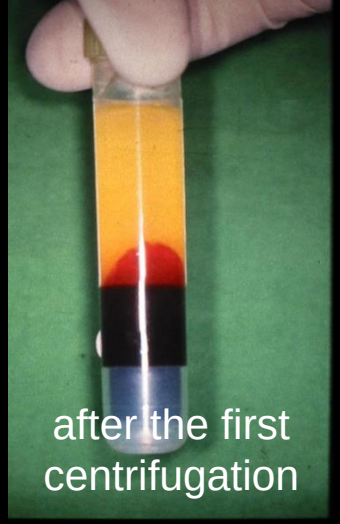
The adjunctive use of platelet-rich plasma in the therapy of periodontal intraosseous defects: a systematic review.

Kotsovilis S, Markou N, Pepelassi E, Nikolidakis D.

J Periodontal Res. 2010 Jun; 45(3):428-43. Epub 2009 Nov 9.

- Aim: The objective of this review was to address the focused question, 'What is the efficacy, with respect to clinical, radiographical and patient-centred outcomes, of combinations of PRP with other therapeutic bioactive agents/procedures, compared with the efficacy of the same agents/procedures without the adjunctive use of PRP in the therapy of periodontal intraosseous defects .
- Conclusion: Diverse outcomes (positive and negative) have been reported for the efficacy of PRP combined with various therapeutic bioactive agents/procedures, reflecting the limited and heterogeneous data available and possibly suggesting that the specific selection of agents/procedures combined with PRP could be important.

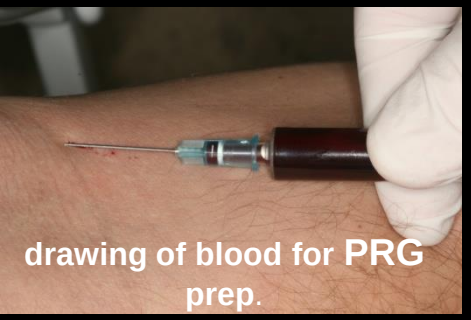

drawing of blood for PRP prep.


after the first centrifugation


after the second centrifugation

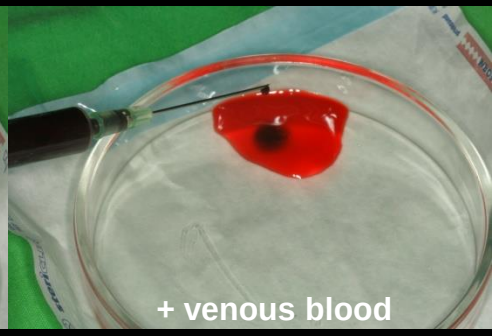

pellet
+ rest plasma

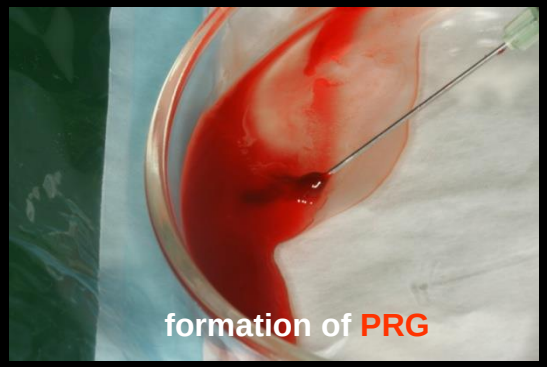
Platelet – Rich Gel preparation

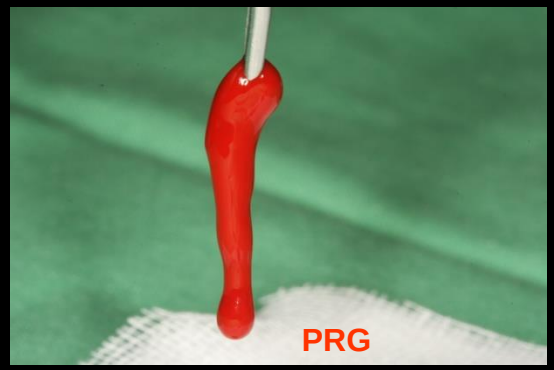

drawing of blood for PRG prep.


Platelet – Rich Plasma


+ Ca-gluconate


+ venous blood


formation of PRG


PRG

F. Dőri, T. Huszár; 2010


THANK YOU