Principais Fixadores e descalcificadores utilizados na rotina e pesquisa:

Dr. Dimitrius Leonardo Pitol

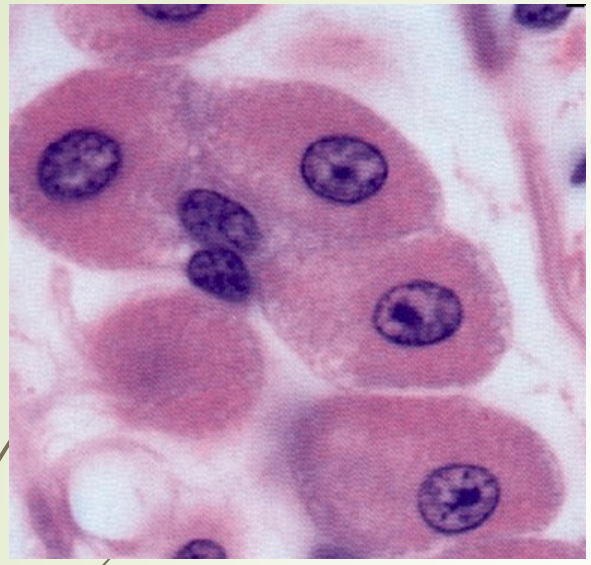
Departamento de Biologia Básica e Oral

Faculdade de Odontologia de Ribeirão Preto

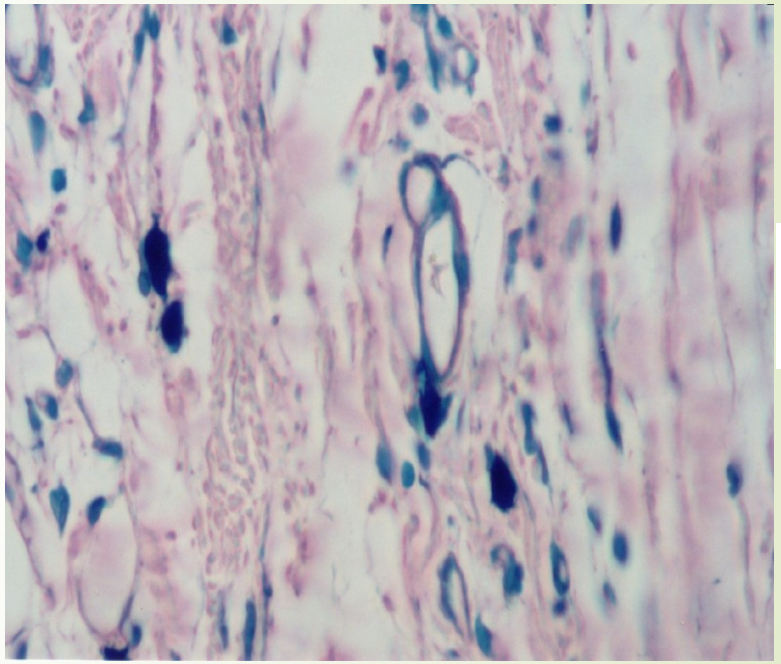
Universidade de São Paulo

Função dos Fixadores

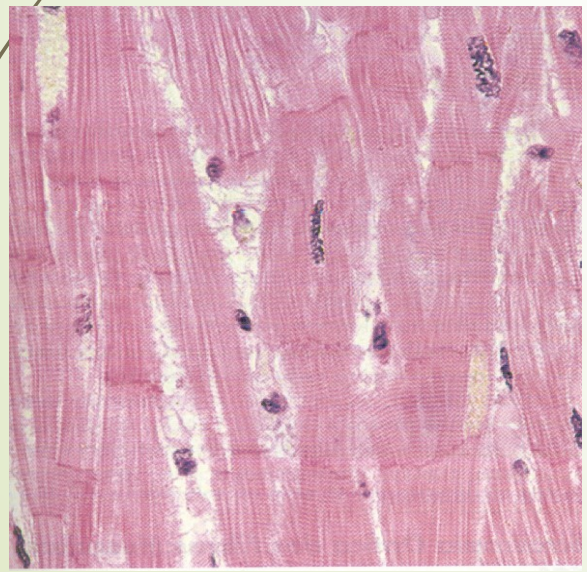
- **Manter a integridade do tecido após a morte.**
- **Evitar o máximo possível Alterações da constituição química da célula.**
- **Fixar proteínas e inativar enzimas proteolíticas mais rapidamente possível, pois são as responsáveis pelo fenômeno de autólise.**



Células Parietais



Derme



Músculo Cardíaco

Fixador ideal

- Penetra na célula ou tecido rapidamente.
- Preserva a estrutura celular antes da célula perder suas características:
- Localização.
- Forma.
- Componentes químicos.

Tipos de fixadores Químicos

Fixadores não aditivos
"coagulantes"

Fixadores adtivos não Coagulantes
(Crosslinking)

Propriedades dos Fixadores Químicos

Coagulantes

Fixadores que precipitam as proteínas

Ex: etanol, metanol, acetona, mercúrio e ácido pícrico

Não Coagulantes

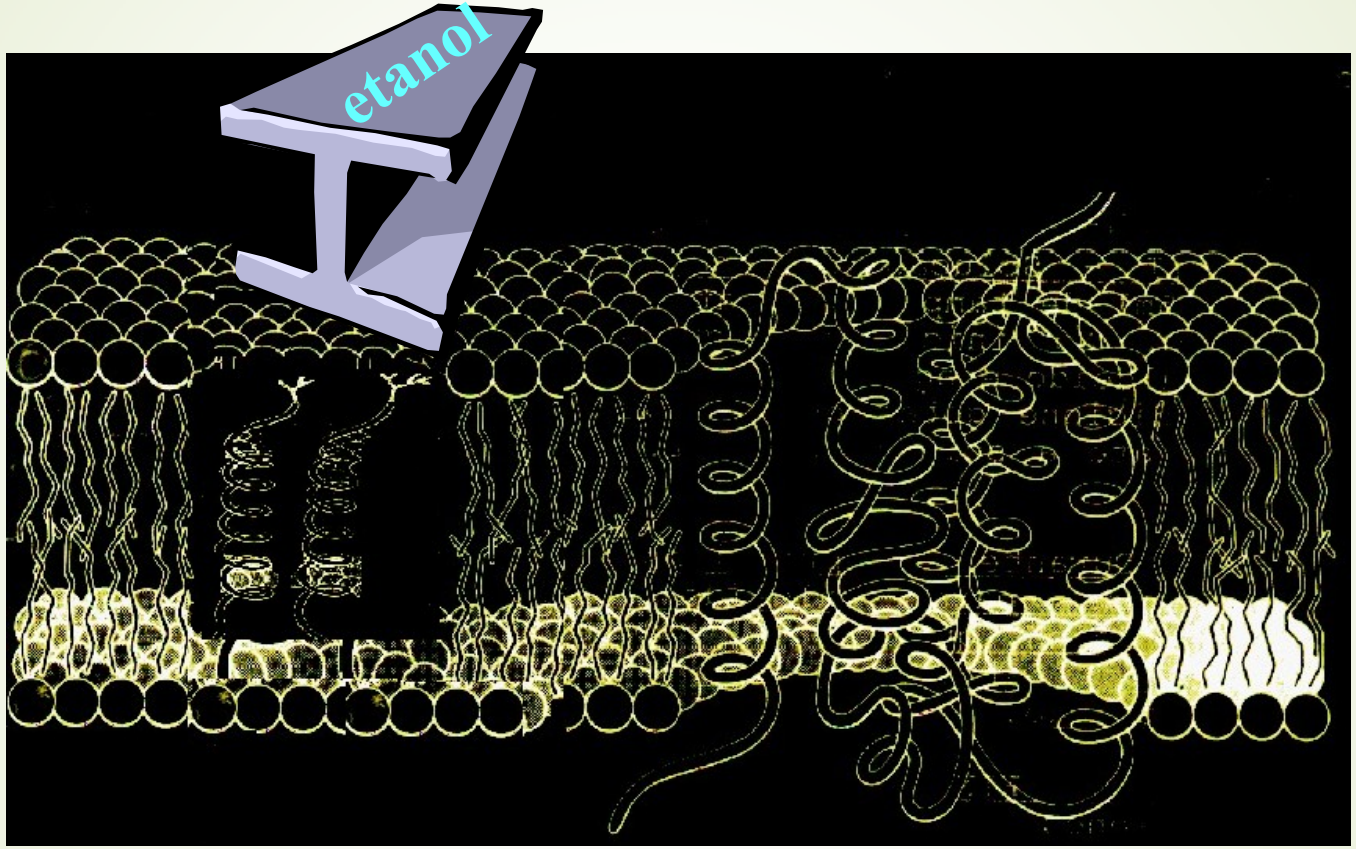
Fixadores que interagem com as proteínas ou seja se ligam a elas

Ex: Formaldeído e paraformaldeído

Ácido Pícrico

- Este fixador fixa as proteínas mas despolimeriza os ácidos nucléicos.
- Sua combinação mais utilizado e ácido pícrico, formol, ácido acético glacial, conhecido como " Bouin".
- Vários pesquisadores preferem esse fixador pora trabalhos com fígado.

Como agem os Fixadores Aditivos "coagulantes?"


etanol

Fixadores Coagulantes

Vantagens:

- Fixa o tecido por uma rápida desidratação dos componentes celulares
- As proteínas são tanto extraídas ou precipitadas.

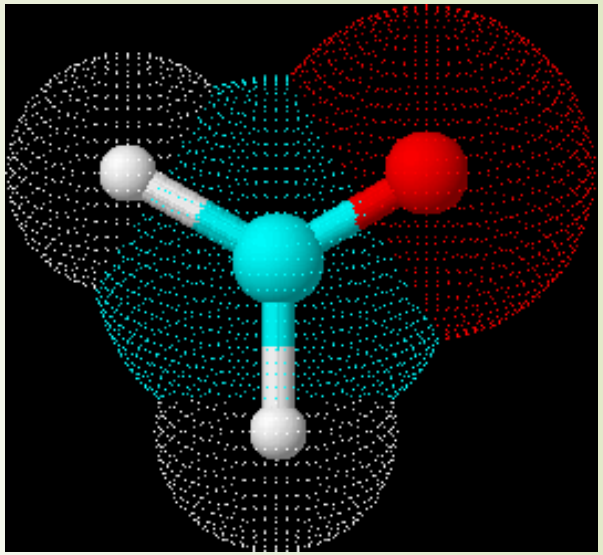
Desvantagens:

- Causa significante encolhimento do tecido.
- Pode encolher cerca de 50% do tamanho das células.

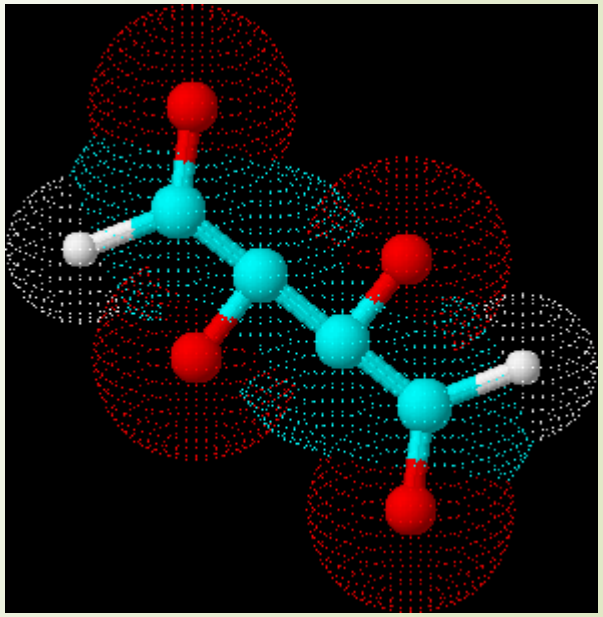
Fixadores Não Coagulantes

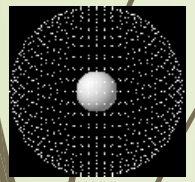
- Paraformoldeido.
- Formoldeido .

Aldeído Fórmico ou formaldeído CH_2O

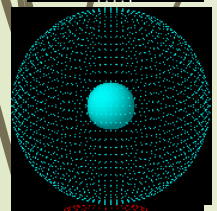


Paraformaldeído ou polioxietileno $(CH_2O)n$

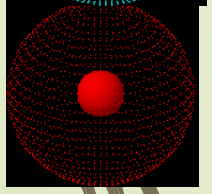




= hidrogênio



= carbono



= oxigênio

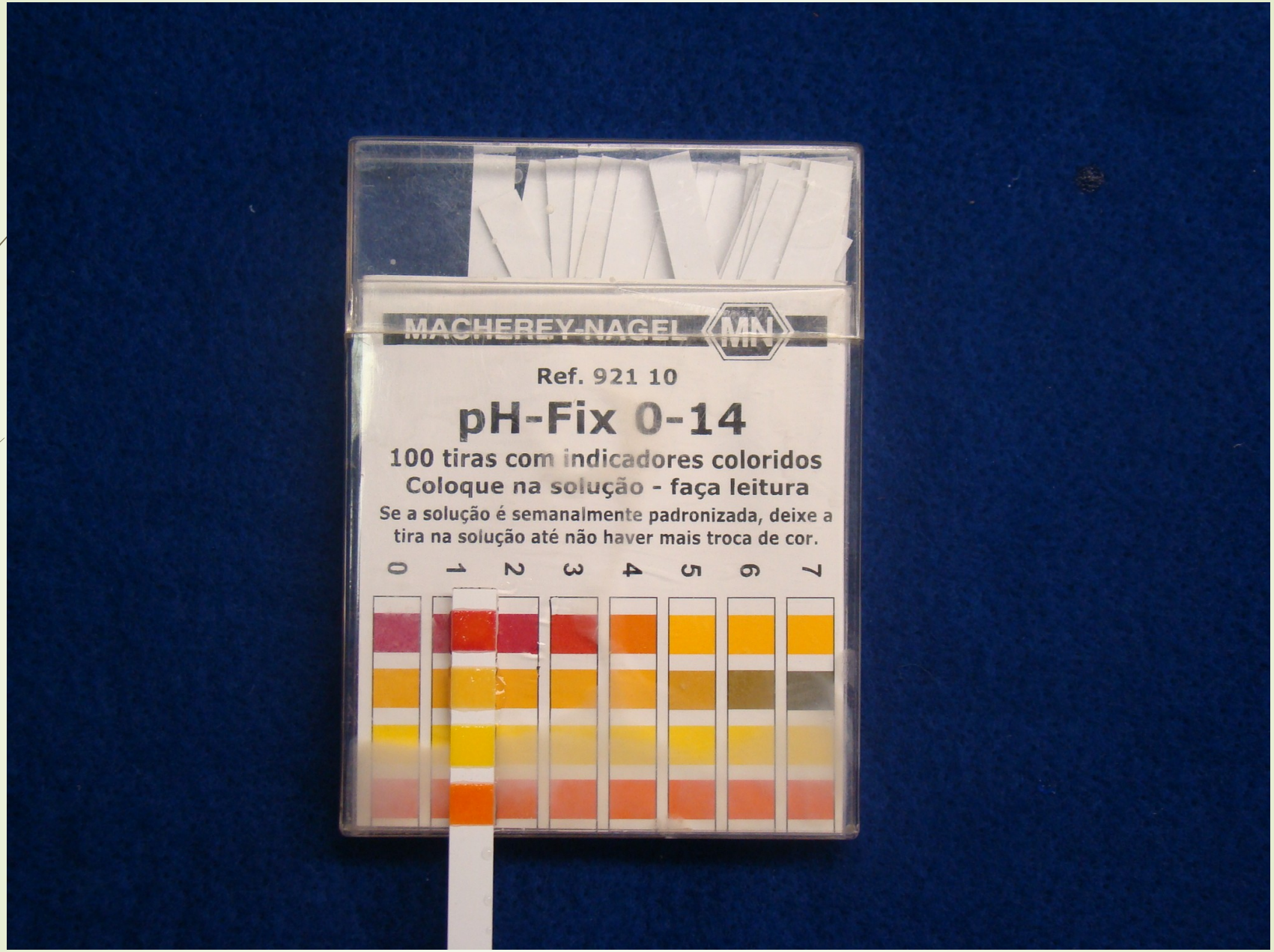
- Como agem os fixadores Não coagulantes?

Aldeído Fórmico

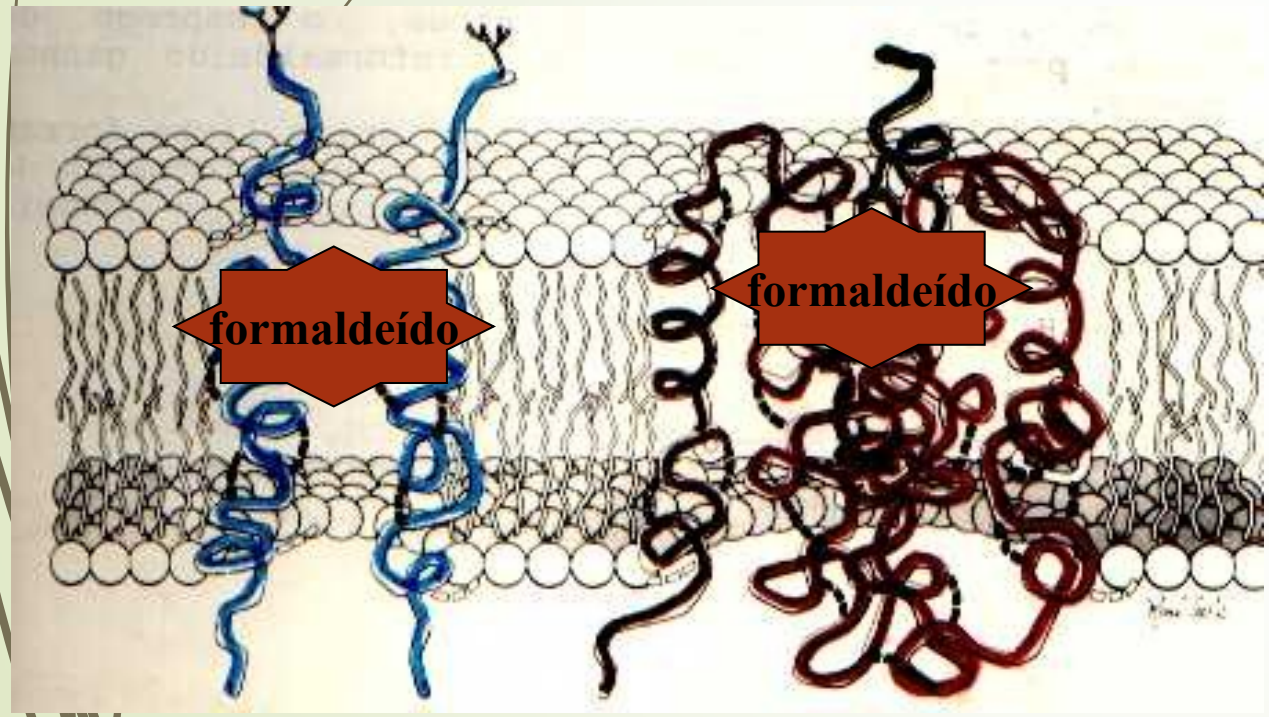
- As células tendem a se separar.
- Mitocôndrios nem sempre são preservados.
- Citoplasma se contrai em relação ao núcleo.
- Preparações comerciais de formaldeído 40% contêm metanol como estabilizante.

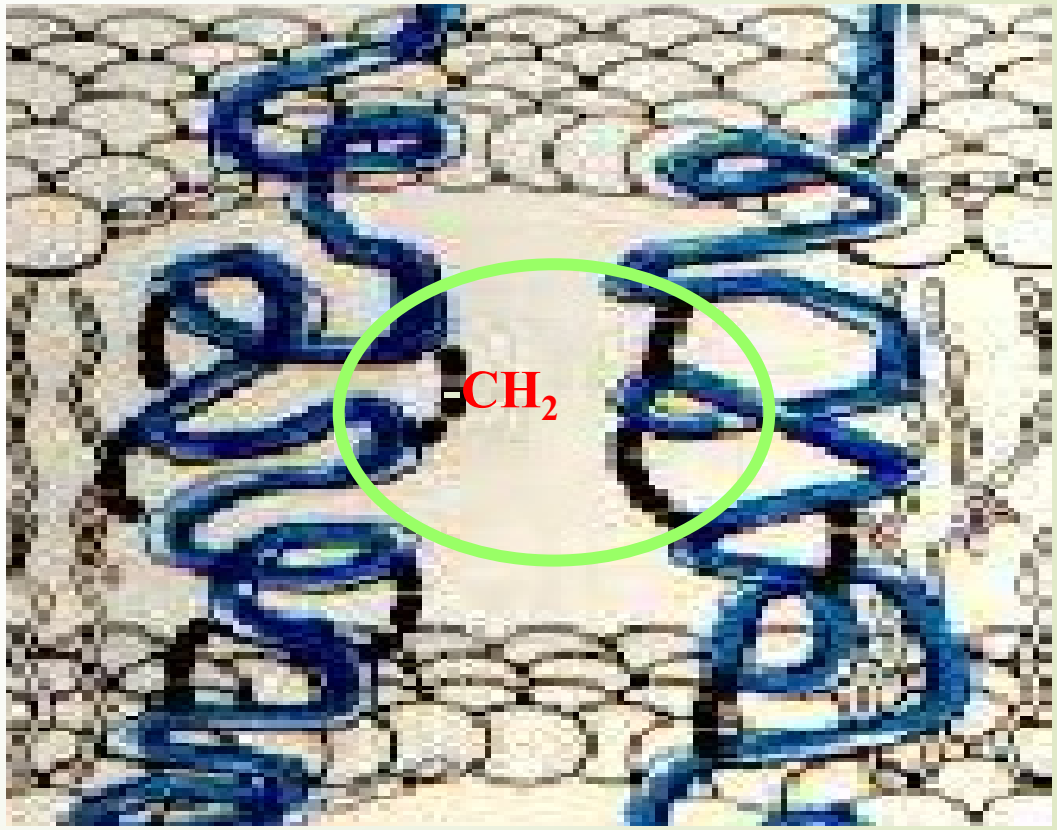

Synth
FORMALDEIDO
SOLUÇÃO
P.A.- ACS
HCHO
P.M. 30,03


FORMALDEIDO
SOLUÇÃO
P.A.- ACS
HCHO
P.M. 30,03

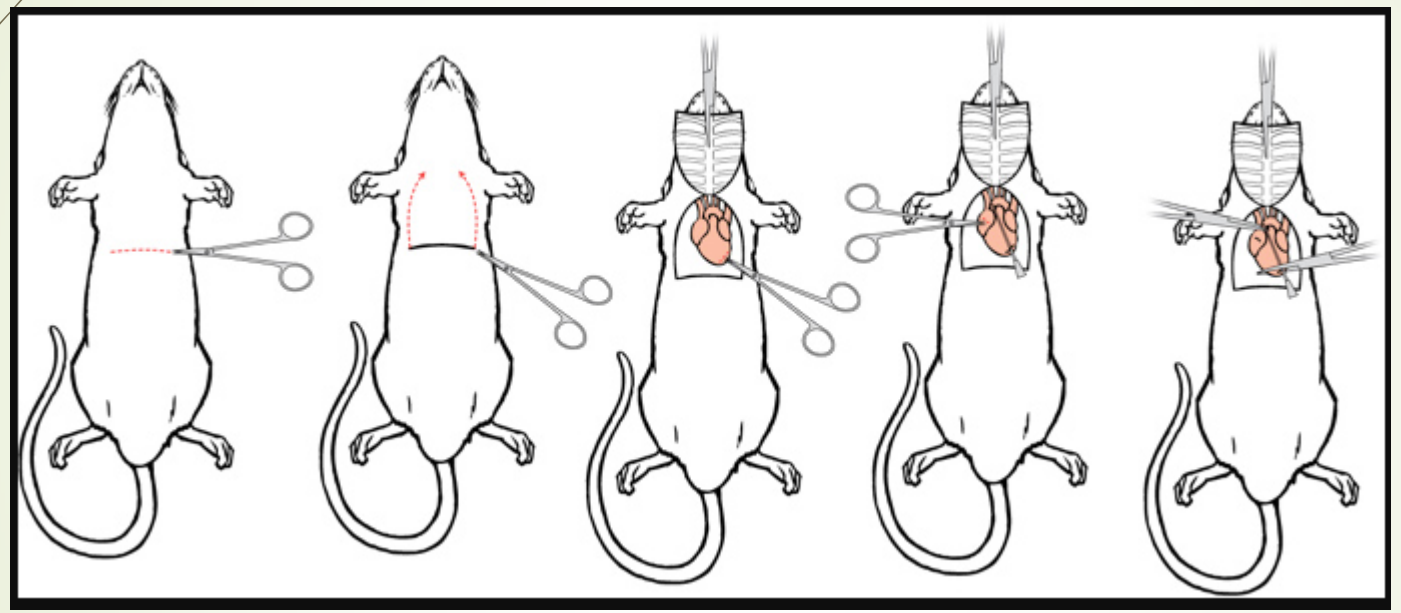

MACHEREY-NAGEL
MN
Ref. 921 10
pH-Fix 0-14
100 tiras com indicadores coloridos
Coloque na solução - faça leitura
Se a solução é semanalmente padronizada, deixe a tira na solução até não haver mais troca de cor.
0 1 2 3 4 5 6 7

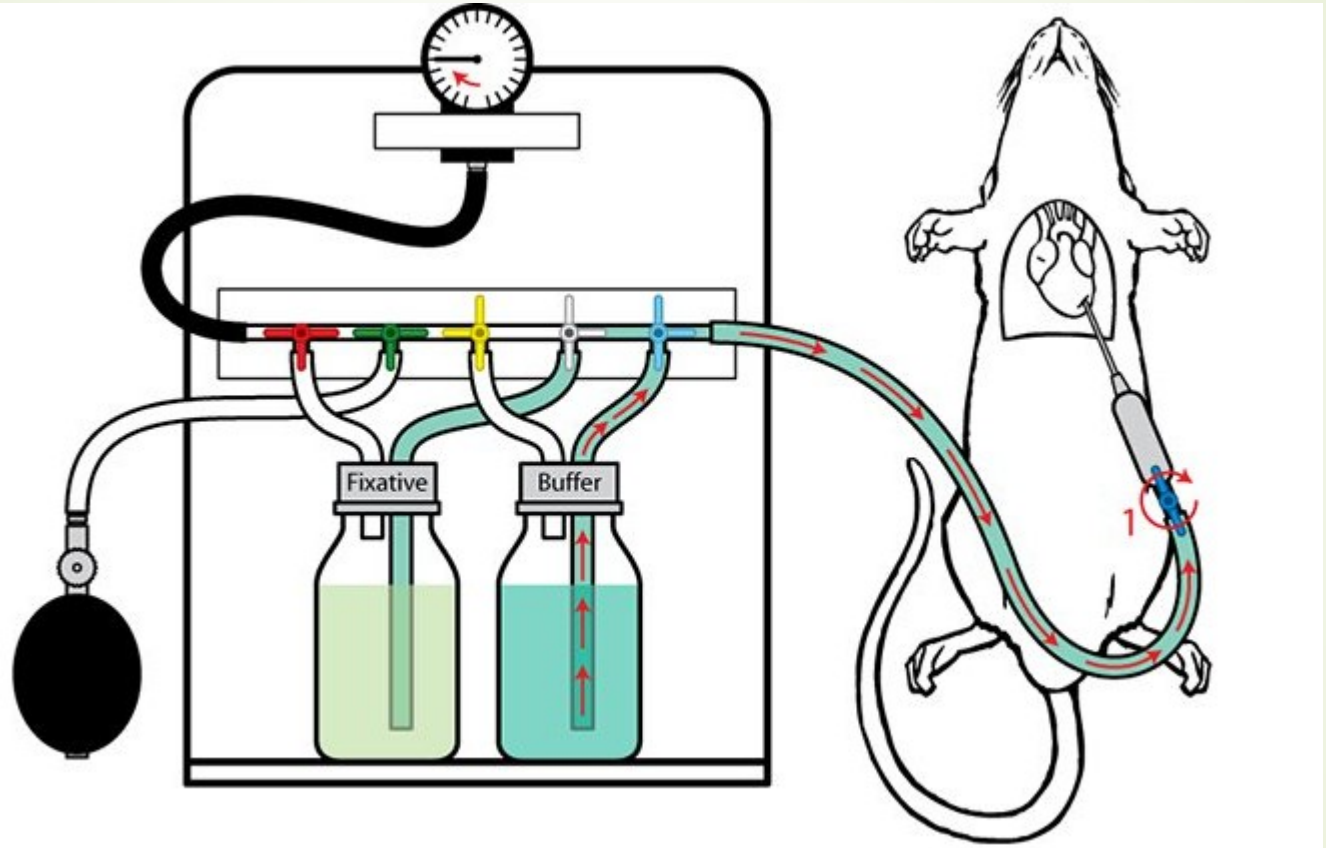
Formam ligações cruzadas do tipo covalente com grupamentos do tecido.





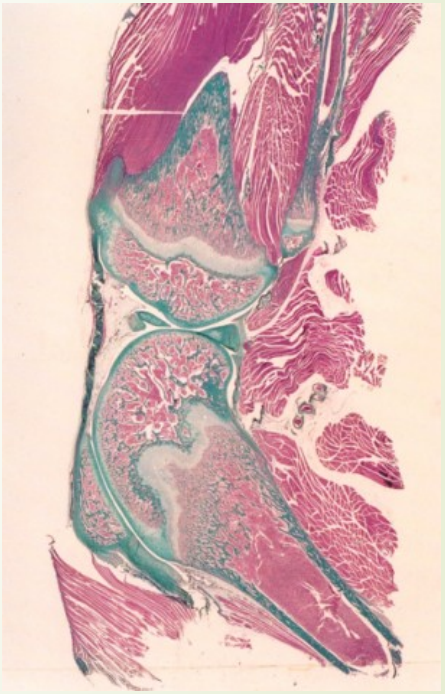
Método de fixação por perfusão




Fixative
Buffer
1

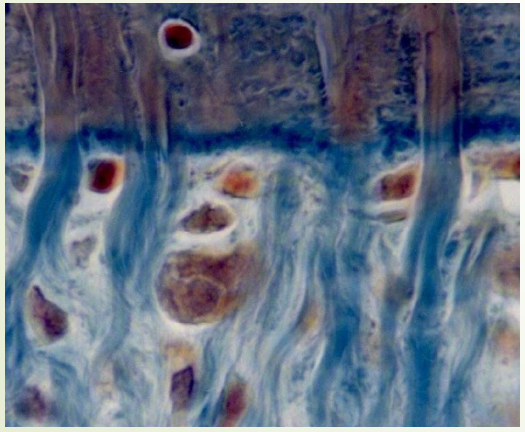
Métodos de descalcificação

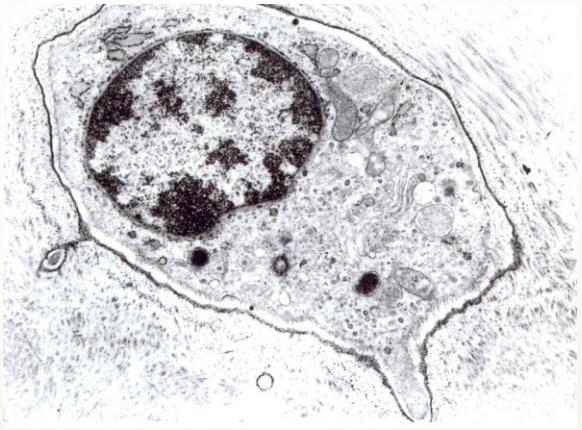
- **Soluções ácidas diluídas.**
- **Soluções quelantes.**
- **Misturas de soluções acidas + soluções quelantes.**



Descalcificadores mais utilizados

- Acido nítrico 5%.
- Acido fórmico + citrato de sódio.
- EDTA (ácido etileno-diamino-tetracético).
- ETDA (tartarato de sódio e potássio, tartarato de sódio Hcl.

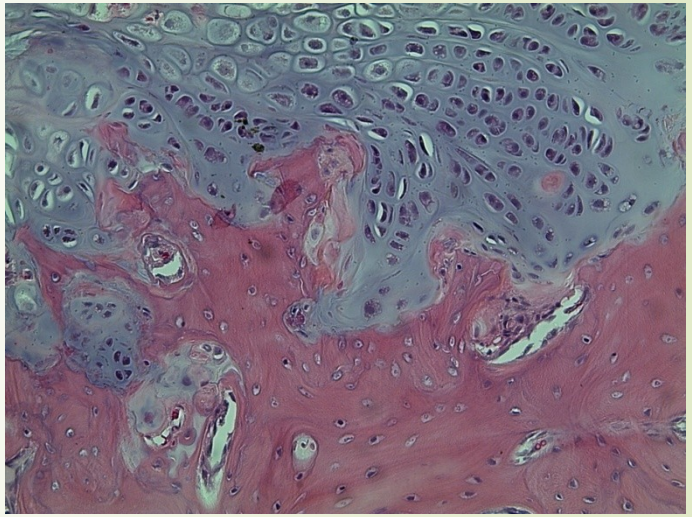




Pitol D.L ;Braz Dent J (2007) 18(2): 153-157

ETDA

- EDTA. 0,7g
- Tartarato de sódio e potássio. 8g
- Tartarato de sódio. 0.14g
- Hcl. 120ml
- H2O destilada. 900ml



MICROSCOPY RESEARCH AND TECHNIQUE 78:111–118 (2015)

Cuidados com a descalcificação

- Material bem fixado.
- Concentração.
- Volume do descalcificador.
- Trocas constantes.
- Ponto Ideal.
- Neutralizar o descalcificador.

Article

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Comparison of Different Decalcification Methods Using Rat Mandibles as a Model

Flavia M. Savi, Gary I. Brierly, Jeremy Baldwin, Christina Theodoropoulos, and Maria A. Woodruff

Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia (FMS, GIB, JB, CT, MAW)

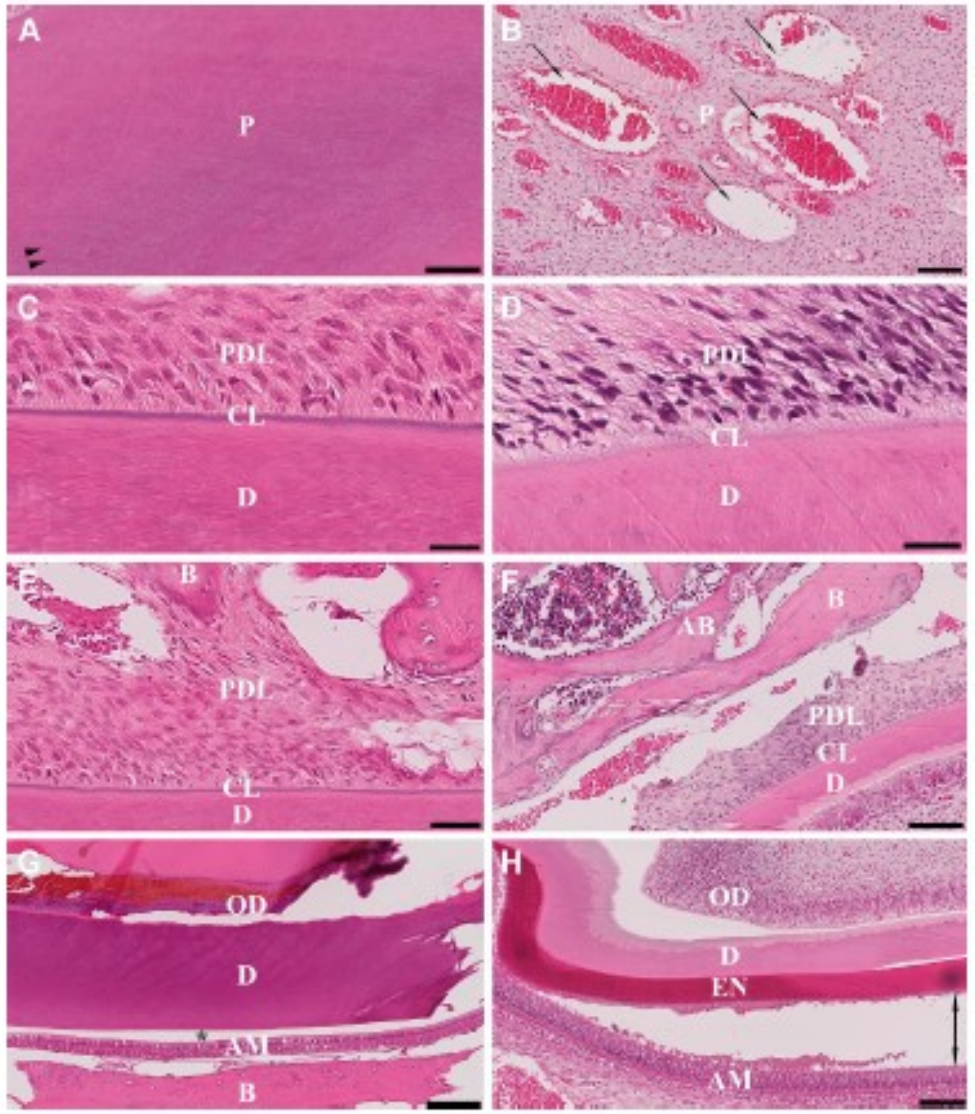


Figure 5. Hematoxylin and eosin-stained sections of the anterior (left column) and posterior (right column) portions of the rat mandible after decalcification with 5% nitric acid. (A, B) Pulp dentinal zone (PDZ); (C, D) cementum line (CL); (E, F) periodontal ligament (PDL); (G, H) dentinoenamel junction (DEJ). Scale bars: A = 200 μm; B, F = 100 μm; C, D = 25 μm; E = 50 μm; G, H = 125 μm. Abbreviations: P, pulp; PDL, periodontal ligament; CL, cementum line; D, dentin; B, bone; AB, alveolar bone; OD, odontoblast layer; AM, ameloblast layer; EN, enamel; one-head arrows, vacuolation; two-head arrows, disruption and cluster of separation of the enamel layer from ameloblast layer; asterisk (*), artifactual space marking the dentinoenamel junction with ameloblast layer; ►►, dentinal tubules.

Acido Nítrico a 5%

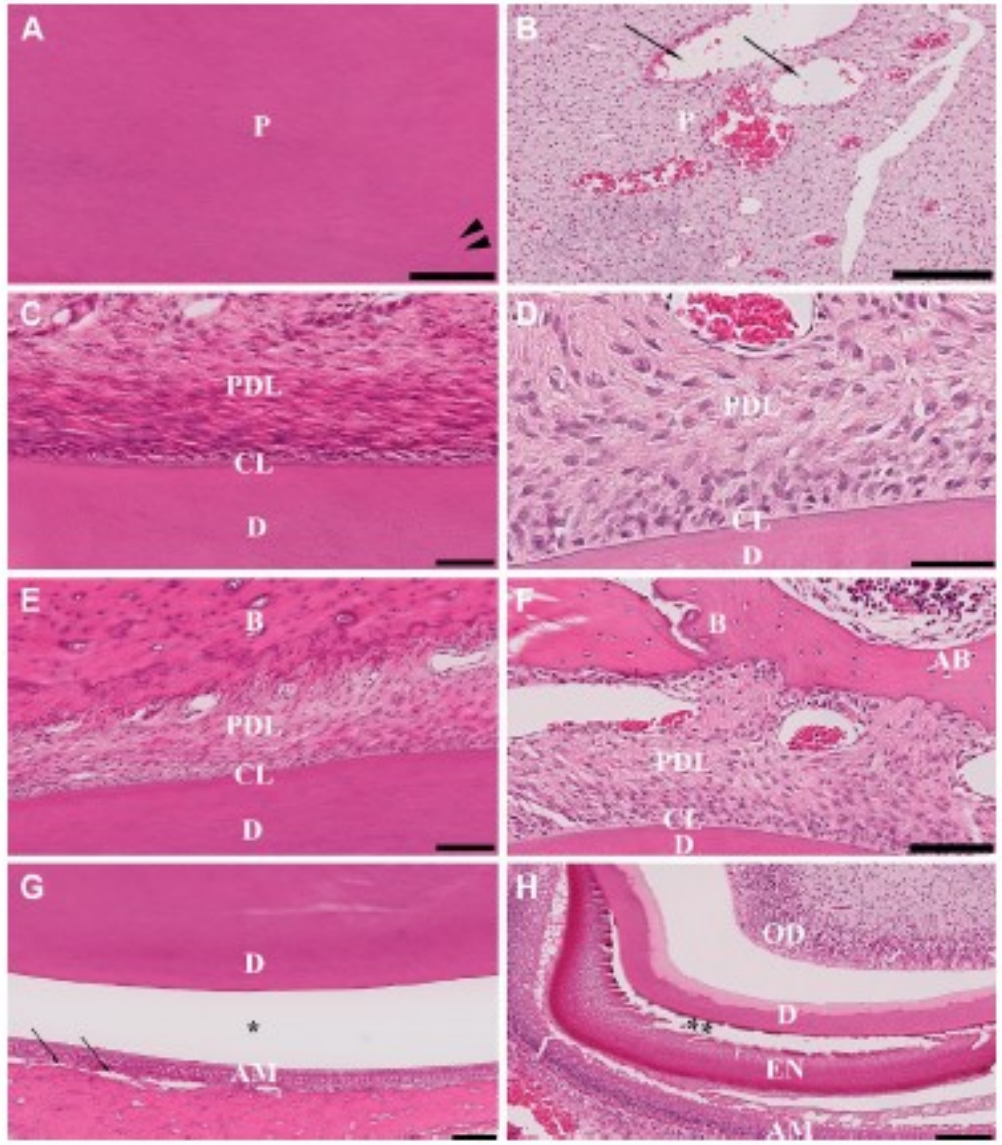


Figure 6. Hematoxylin and eosin–stained sections of the anterior (left column) and posterior (right column) portions of the rat mandible after decalcification with 10% formic acid. (A, B) Pulp dentinal zone (PDZ); (C, D) cementum line (CL); (E, F) periodontal ligament (PDL); (G, H) dentinoenamel junction (DEJ). Scale bars: A, B = 200 µm; C, D = 50 µm; E, F = 100 µm; G = 250 µm; H = 125 µm. Abbreviations: P, pulp; PDL, periodontal ligament; CL, cementum line; D, dentin; B, bone; AB, alveolar bone; AM, ameloblast layer; OD, odontoblast layer; EN, enamel; one-head arrows, vacuolation; asterisk (*), artifactual space marking the dentinoenamel junction with

Acido fórmico

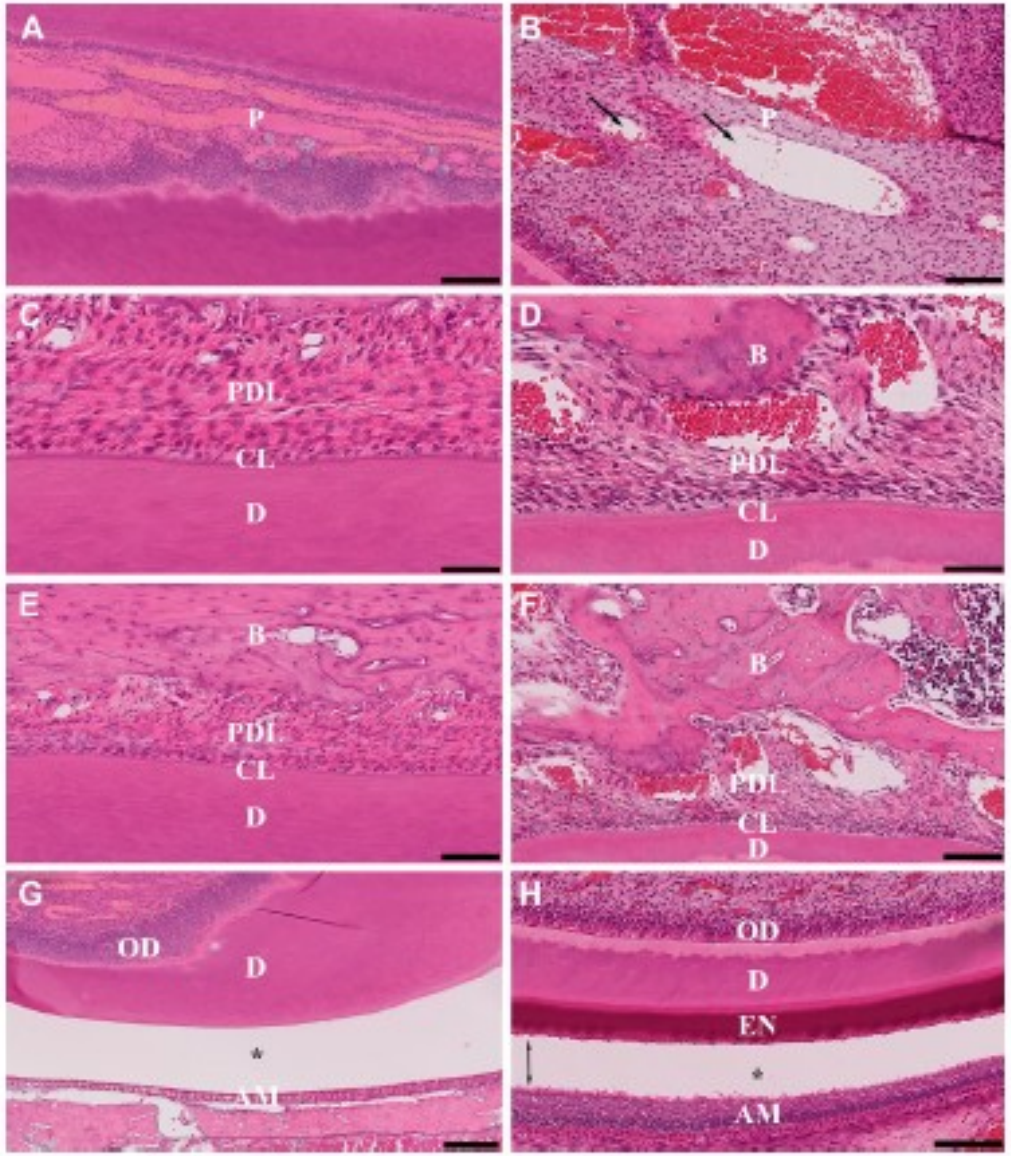


Figure 3. Hematoxylin and eosin–stained sections of the anterior (left column) and posterior (right column) portions of the rat mandible after decalcification with 10% EDTA RT. (A, B) Pulp dentinal zone (PDZ); (C, D) cementum line (CL); (E, F) periodontal ligament (PDL); (G, H) dentinoenamel junction (DEJ). Scale bars: A = 100 μm; B, E, F = 100 μm; C, D = 50 μm; G = 250 μm; H = 125 μm. Abbreviations: EDTA, ethylenediaminotetraacetic acid; RT, room temperature; P, pulp; D, dentin; B, bone; OD, odontoblast layer; AM, ameloblast layer; EN, enamel; one-head arrows, vacuolation; two-head arrows, disruption of the enamel layer from surrounding ameloblast layer; asterisk (*), artifactual space marking the dentinoenamel junction with ameloblast layer.

EDTA 10%

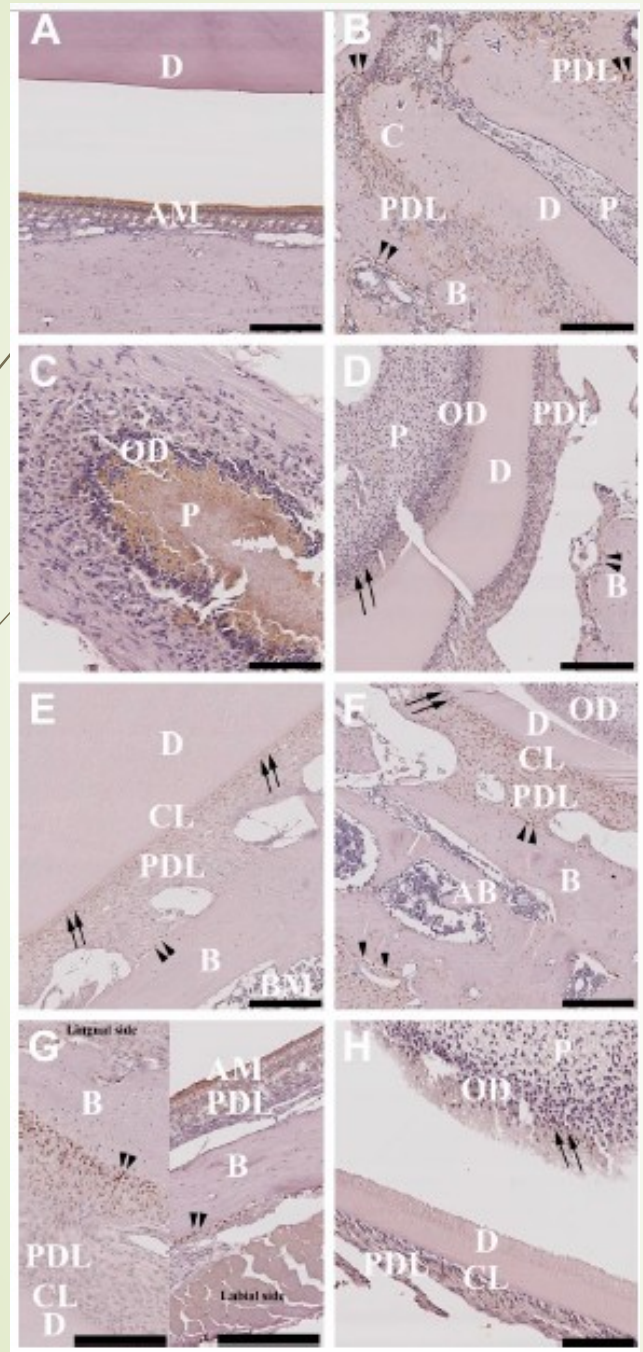


Figure 9. Immunolocalization of Osx in the anterior (left column) and posterior (right column) portions of the rat mandible following use of varying decalcification agents. Decalcification with 10% EDTA (A, B); 10% EDTA at 37C (C, D); 5% nitric acid (E, F); 10% formic acid (G, H). (A) Dentinoenamel junction (DEJ); (B) molar's root complex; (C, D, H) pulp dentinal zone (PDZ); (E, F) periodontal ligament complex (PDL); (G) periodontal ligament complex (PDL) and dentinoenamel junction (DEJ). Negative control rabbit IgG was used to label adjacent tissue sections for each decalcification agent (Supplementary Fig. 3A, C, E, G [anterior]; B, D, F, H [posterior]). Scale bars: A–F, H = 200 μm; C, G = 100 μm. Abbreviations: Osx, osterix; EDTA, ethylenediaminetetraacetic

(continued)

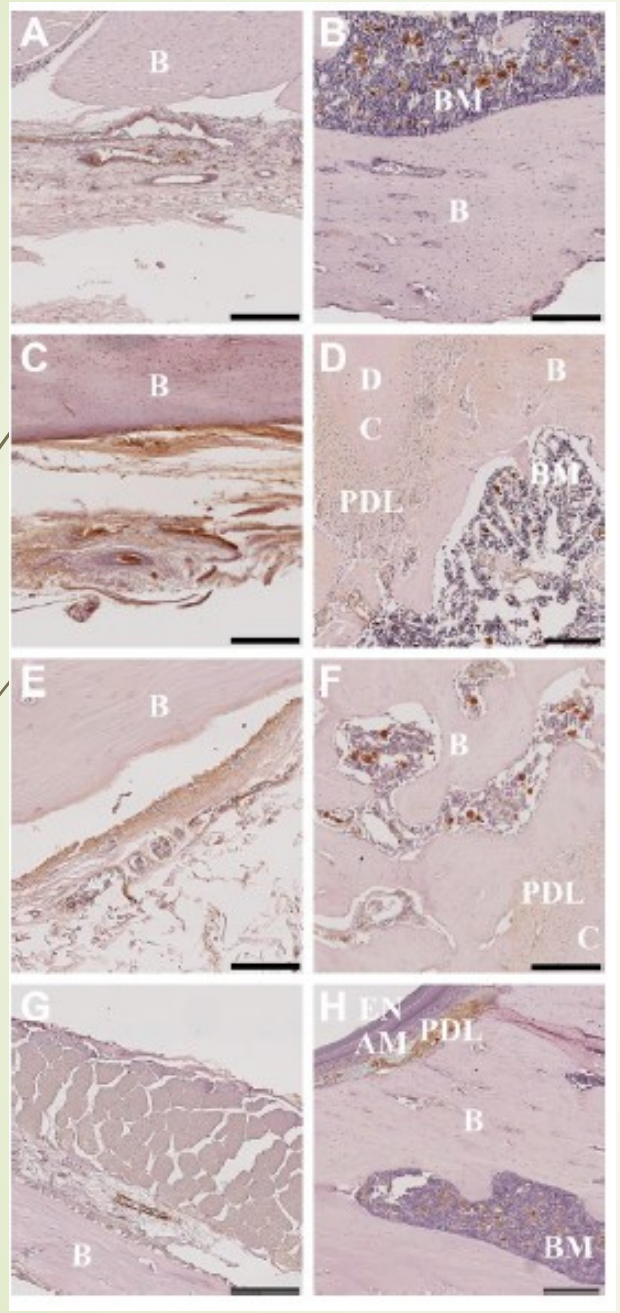


Figure 7. Immunolocalization of vWF in the anterior (left column) and posterior (right column) portions of the rat mandible following the use of varying decalcification agents. Decalcification with 10% EDTA at room temperature (A, B); 10% EDTA at 37C (C, D); 5% nitric acid (E, F); 10% formic acid (G, H). Negative control rabbit IgG was used to label adjacent tissue sections for each decalcification agent (Supplementary Fig. 1A, C, E, G [anterior]; B, D, F, H [posterior]). Scale bars: A–D, F, H = 200 μm; E, G = 100 μm. Abbreviations: vWF, von Willebrand factor; EDTA, ethylenediaminetetraacetic acid; B, bone; BM, bone marrow; D, dentin; C, cementum; B, bone; PDL, periodontal ligament; EN, enamel; AM, ameloblast layer.

25. Mori S, Sawai T, Teshima T, Kyogoku M. A new decalcifying technique for immunohistochemical studies of calcified tissue, especially applicable to cell surface marker demonstration. J Histochem Cytochem. 1988;36(1):111–4. doi:10.1177/36.1.3275709.
26. Bourque WT, Gross M, Hall BK. A histological processing technique that preserves the integrity of calcified tissues (bone, enamel), yolky amphibian embryos, and growth factor antigens in skeletal tissue. J Histochem Cytochem. 1993;41(9):1429–34.
27. Frank JD, Balena R, Masarachia P, Seedor JG, Cartwright ME. The effects of three different demineralization agents on osteopontin localization in adult rat bone using immunohistochemistry. Histochemistry. 1993;99(4):295–301.
28. Castania VA, Silveira JW, Issy AC, Pitol DL, Castania ML, Neto AD, Bel EA, Defino HL. Advantages of a combined method of decalcification compared to EDTA. Microsc Res Tech. 2015;78(2):111–8. doi:10.1002/jemt.22451.
29. Schajowicz F, Cabrini RL. The effect of acids (decalcifying solutions) and enzymes on the histochemical behavior of bone and cartilage. J Histochem Cytochem. 1955;3(2):122–9. doi:10.1177/3.2.122.
30. Verdenius HHW, Alma L. A quantitative study of decalcification methods in histology. J Clin Pathol. 1958;11(3):229–36. doi:10.1136/jcp.11.3.229.
31. Pitol DL, Caetano FH, Lunardi LO. Microwave-induced fast decalcification of rat bone for electron microscopic analysis: an ultrastructural and cytochemical study. Braz Dent J. 2007;18(2):153–7.
37. Goldberg M, Kellermann O, Dimitrova-Nakov S, Harichane Y, Baudry A. Comparative studies between mice molars and incisors are required to draw an overview of enamel structural complexity. Front Physiol. 2014;5:359. doi:10.3389/fphys.2014.00359.
38. Prasad P, Donoghue M. A comparative study of various decalcification techniques. Indian J Dent Res. 2013;24(3):302–8. doi:10.4103/0970-9290.117991.
39. Farrell SF, Osmotherly PG, Cornwall J, Rivett DA. The anatomy and morphometry of cervical zygapophyseal joint meniscoids. Surg Radiol Anat. 2015;37(7):799–807. doi:10.1007/s00276-014-1406-3.
40. Bussolati G, Leonardo E. Technical pitfalls potentially affecting diagnoses in immunohistochemistry. J Clin Pathol. 2008;61:1184–92. doi:10.1136/jcp.2007.047720.
41. Athanasou NA, Quinn J, Heryet A, Woods CG, McGee JO. Effect of decalcification agents on immunoreactivity of cellular antigens. J Clin Pathol. 1987;40(8):874–8. doi:10.1136/jcp.40.8.874.
42. González-Chávez SA, Pacheco-Tena C, Macías-Vázquez CE, Luévano-Flores E. Assessment of different decalcifying protocols on Osteopontin and Osteocalcin immunostaining in whole bone specimens of arthritis rat model by confocal immunofluorescence. Int J Clin Exp Pathol. 2013;6(10):1972–83.
43. Herrington CS. Muir's textbook of pathology. 15th ed. Boca Raton, FL: CRC Press; 2014.
44. Kiviranta, I, Tammi, M, Lappalainen, R, Kuusela, T, Helminen, HJ. The rate of calcium extraction during EDTA decalcification from thin bone slices as assessed

EDTA

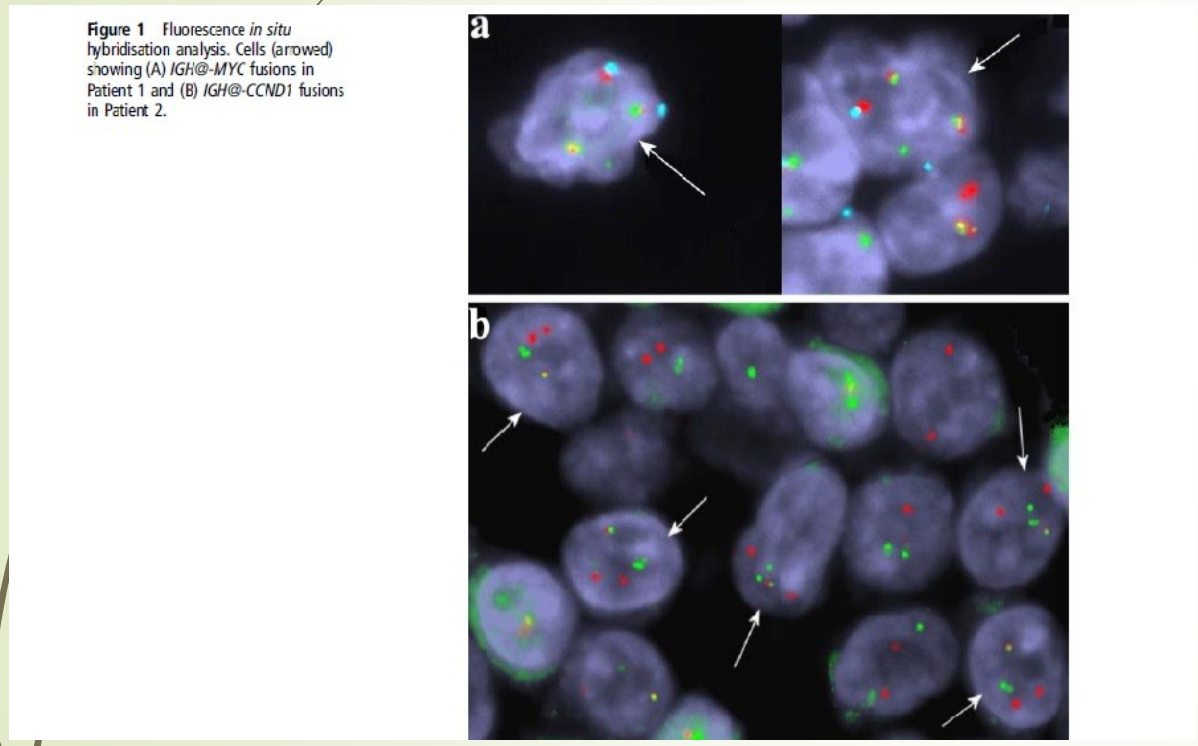


Figure 1 Fluorescence *in situ* hybridisation analysis. Cells (arrowed) showing (A) *IGH@-MYC* fusions in Patient 1 and (B) *IGH@-CCND1* fusions in Patient 2.

- Entretanto os protocolos com EDTA em biopsia de medula variam de 48 a 72 horas

Neat MJ, et al. J Clin Pathol 2013;66:54–57.

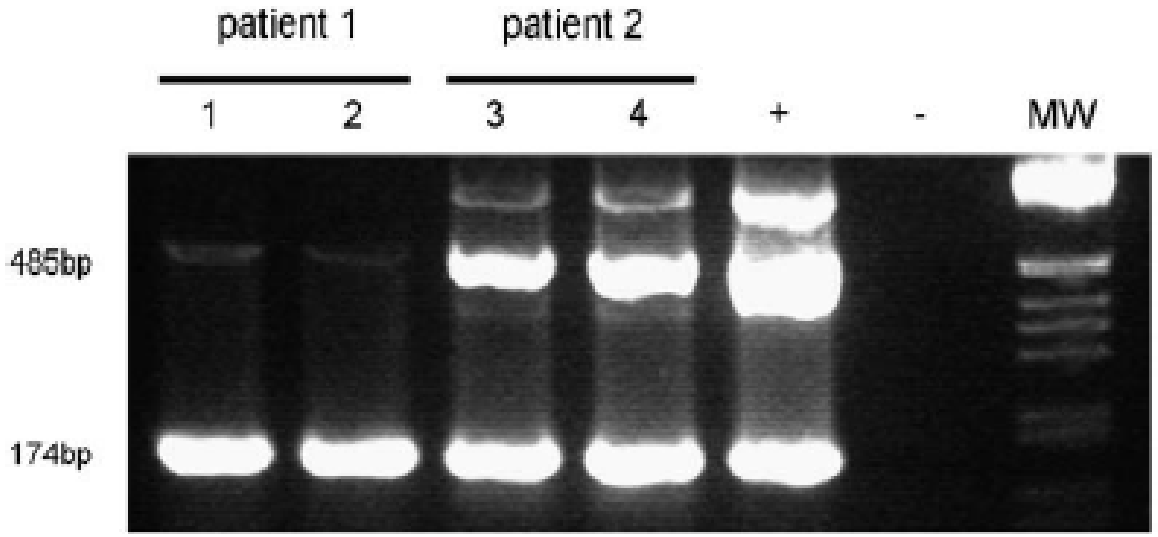
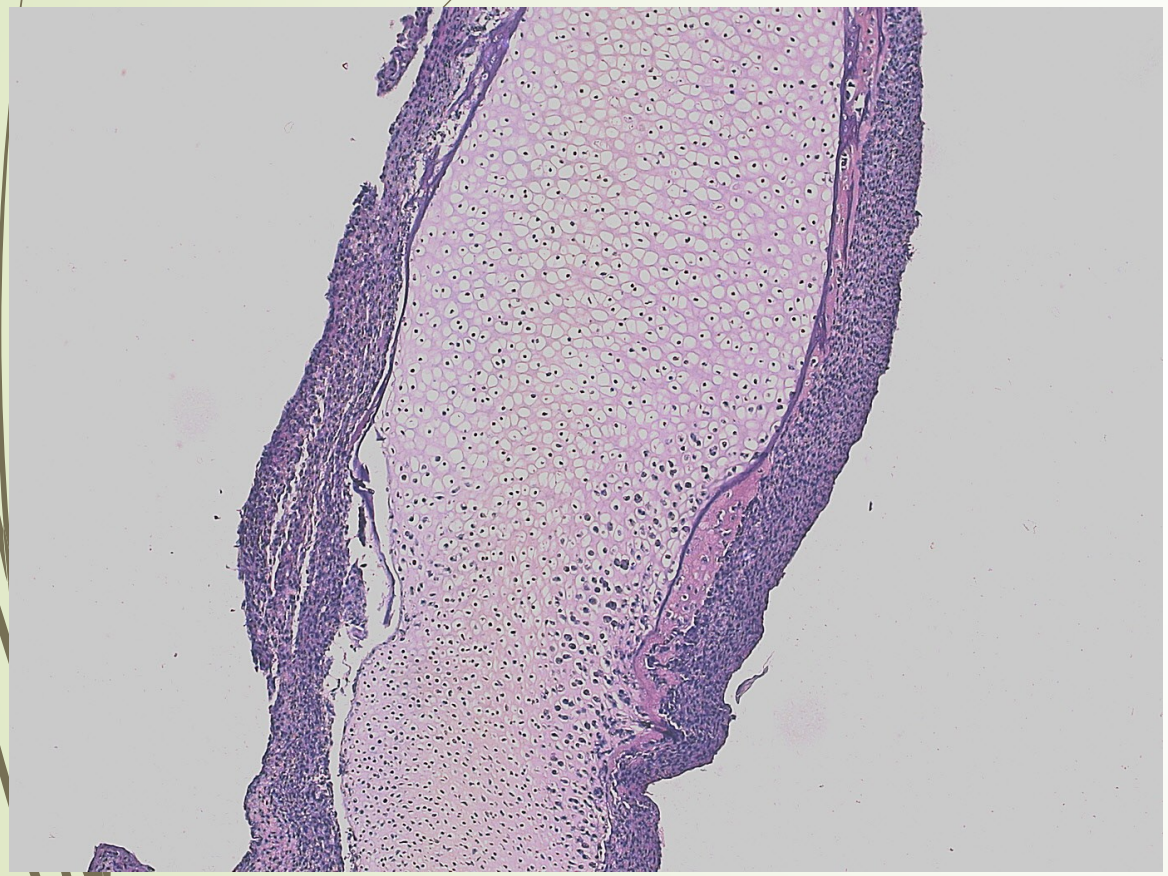
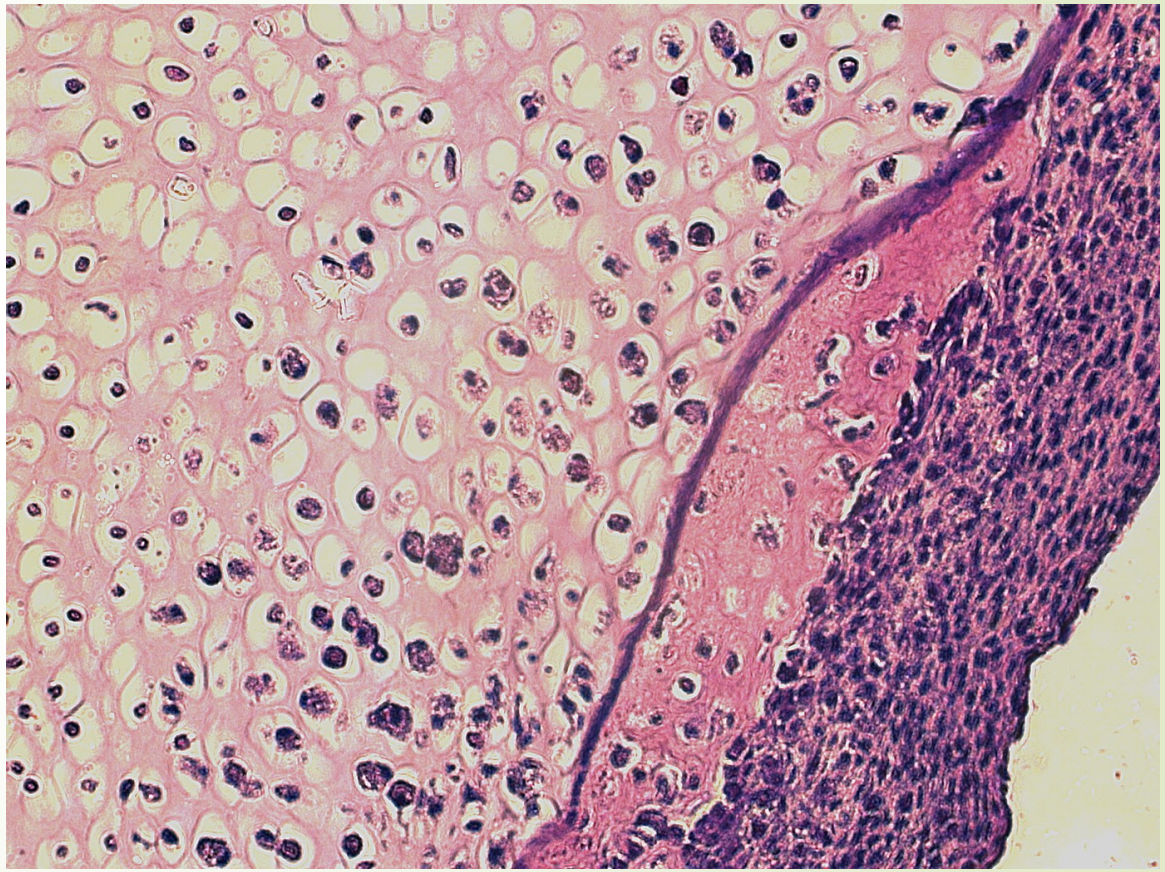


FIGURE 6. Samples of 2 patients (patient 1: No. 1 and 2; patient 2: No. 3 and 4) fixed in 4% formalin for 24 hours and decalcified by ultrasound and EDTA-based solutions showing variable amounts of amplified RNA for β-actin. No. 1 and 3 = USEDECALC; No. 2 and 4 = 10% EDTA. RNA from paraffin-embedded tissue as PCR positive control. – water as negative control.





Lasers Med Sci (2015) 30:1599–1607
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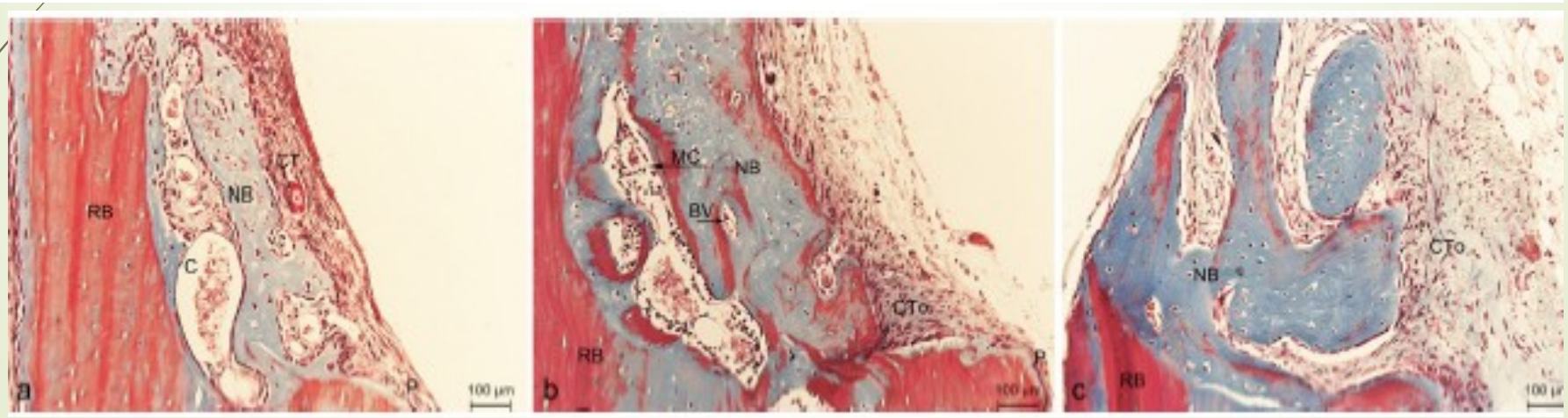
ORIGINAL ARTICLE

Low-level laser therapy improves bone formation: stereology findings for osteoporosis in rat model

Priscilla Hakime Scalize[1] · Luiz Gustavo de Sousa[1] · Simone Cecílio Hallak Regalo[1] · Marisa Semprini[1] · Dimitrius Leonardo Pitol[1] · Giselle Aparecida da Silva[1] · Jéssica de Almeida Coelho[1] · Antônio Augusto Coppi[2] · Aliny A B Lobo Laad[3] · Karina Fittipaldi Bombonato Prado[1] · Selma Siessere[1]



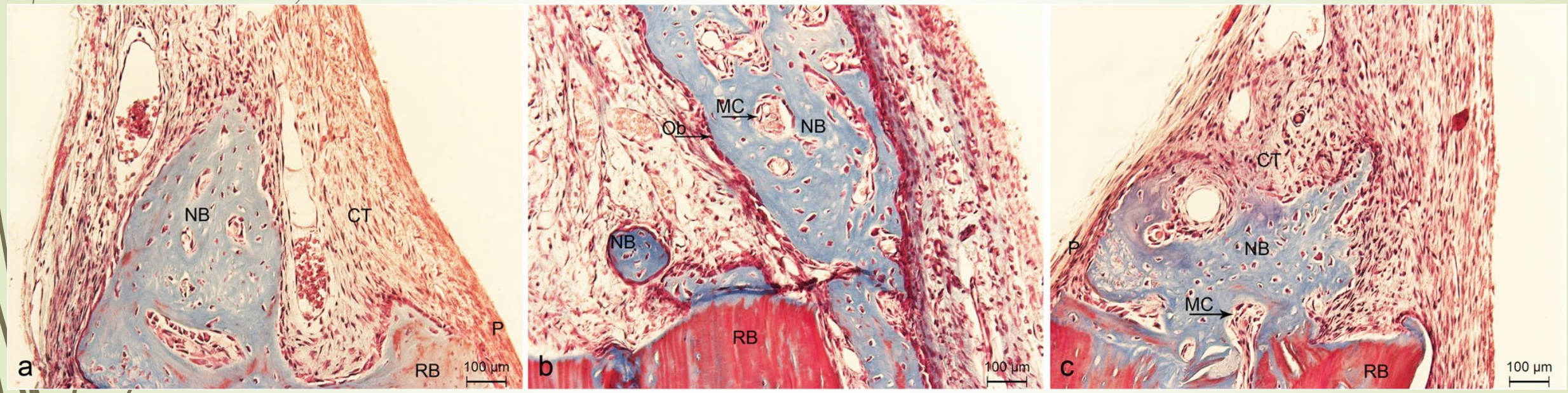
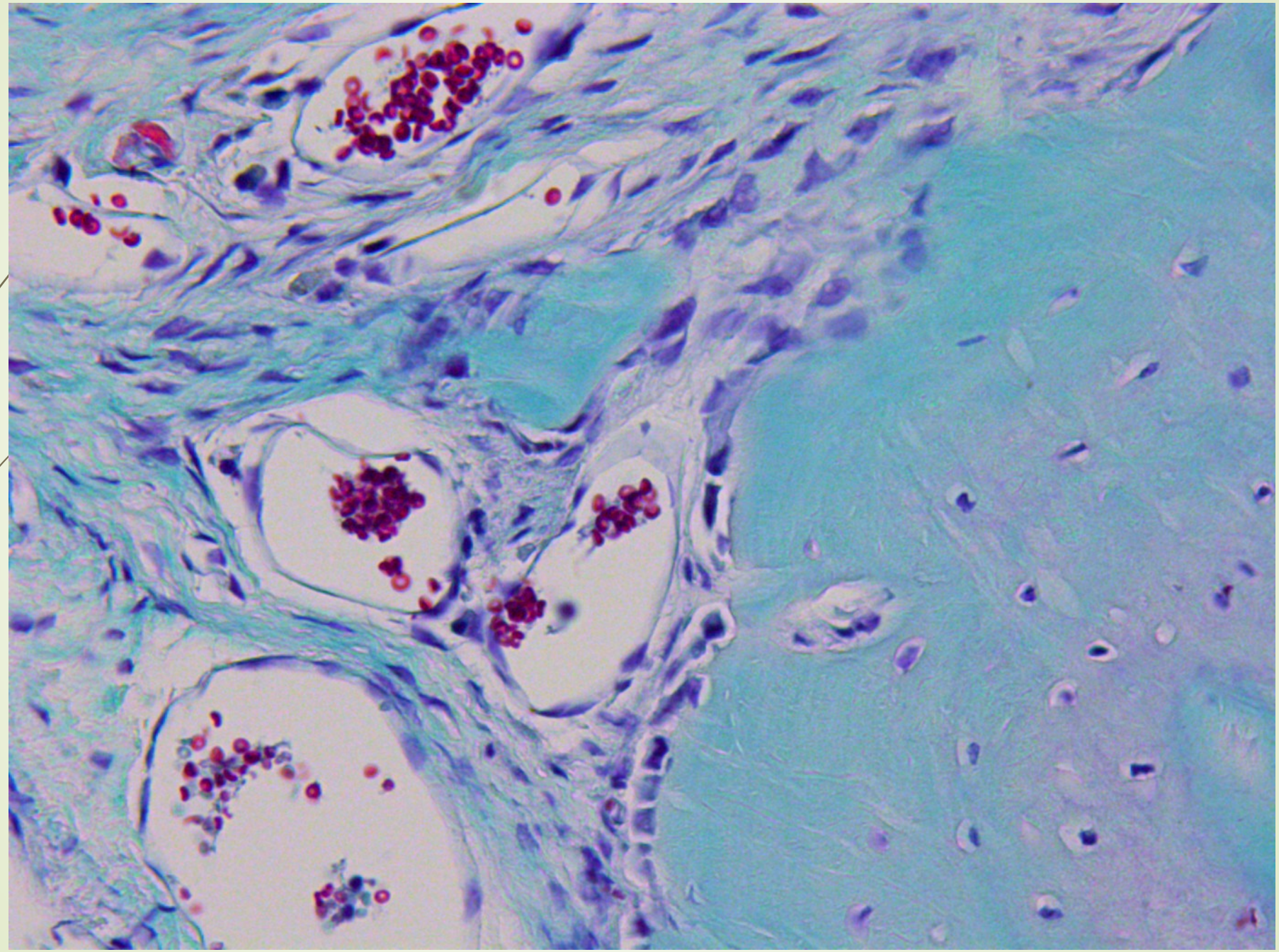


Fig. 2 Photomicrographs of the groups that received 6 laser sessions. In a G3, 0 J/cm$_2$; b G4, 20 J/cm$_2$; and c G5, 30 J/cm$_2$. Periphery of the bone defect
region. NB newly formed bone, CTconnective tissue, P periosteum, MCmedullary cavity, Ob osteoblasts, RB remaining bone. Masson's trichrome stain



Scale morphology of *Prochilodus lineatus* with emphasis on the scale epithelium

Alves, RMS.[a]*, Pereira, BF.*[a]*, Pitol, DL.*[b]*, Senhorini, JA.*[c]*, Alcântara-Rocha, RCG.*[c] *and Caetano, FH.*[a]

[a]*Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências, Campus de Rio Claro, Av. 24A, 1515, Bela Vista, CEP 13506-900, Rio Claro, SP, Brazil*
[b]*Faculdade de Odontologia, Universidade de São Paulo - USP, Ribeirão Preto, SP, Brazil*
[c]*Laboratório de Reprodução de Peixes - ICMBio/CEPTA, Pirassununga, SP, Brazil*
[*]*e-mail: bella_reis@hotmail.com*

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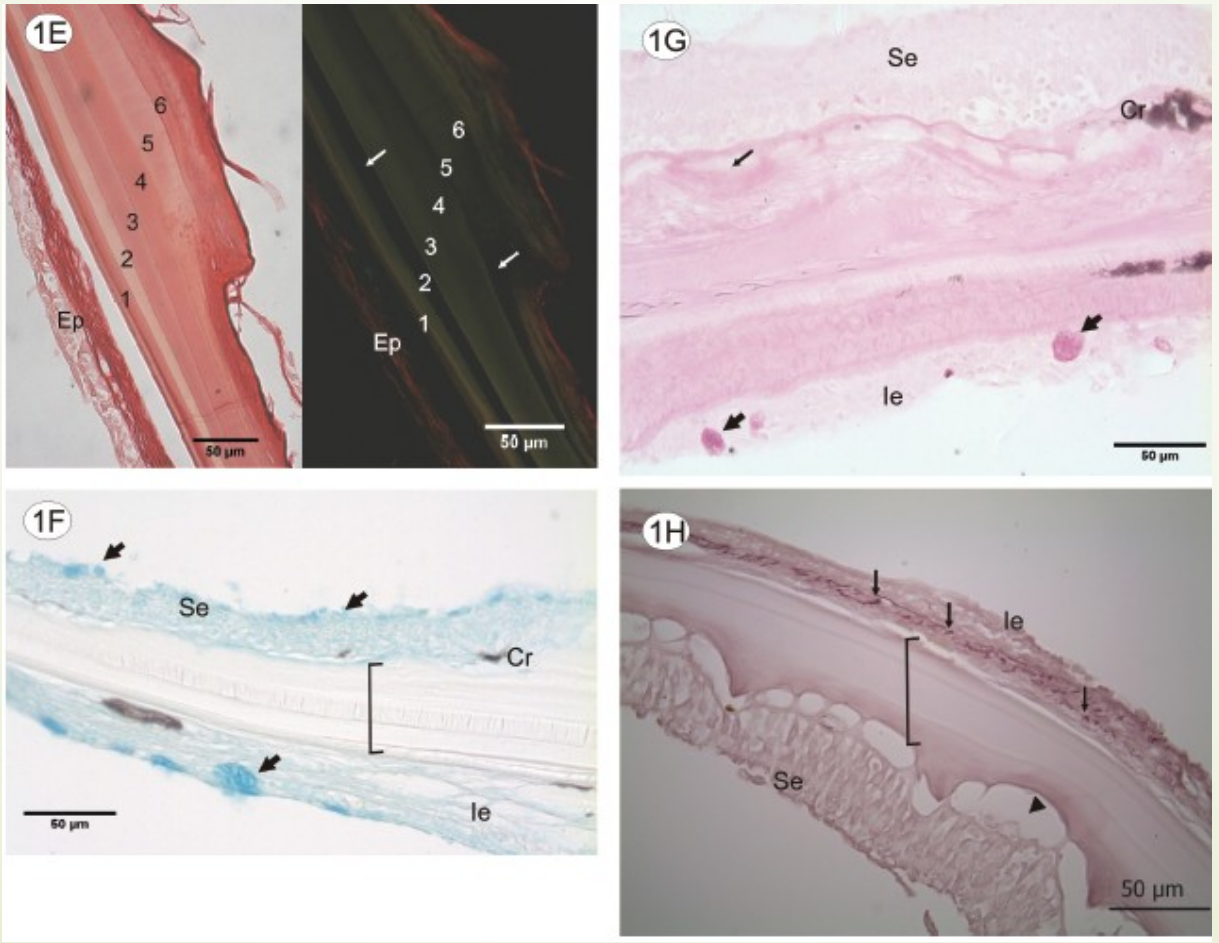

Agradecimentos

dimitrius@forp.usp.br
dimipitol@yahoo.com.br