

Métodos de Descalcificação

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Processamento de Tecidos mineralizados



COROA

POLPA

DENTINA

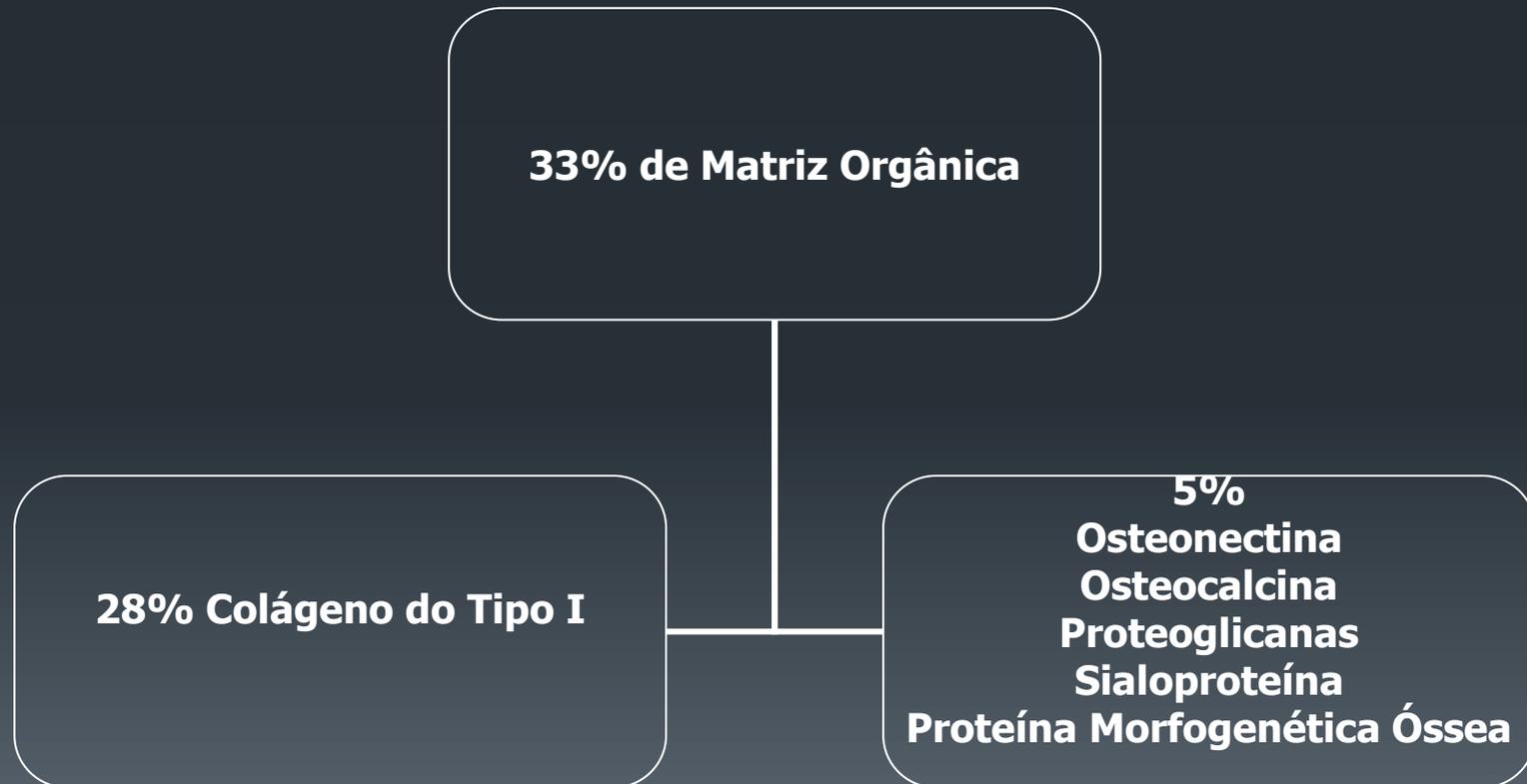


**Osso Trabeculado
ou Esponjoso**

**Medula Óssea
vermelha**

**Osso Cortical
ou
Compacto ou
Denso**

Tecido Mineralizado



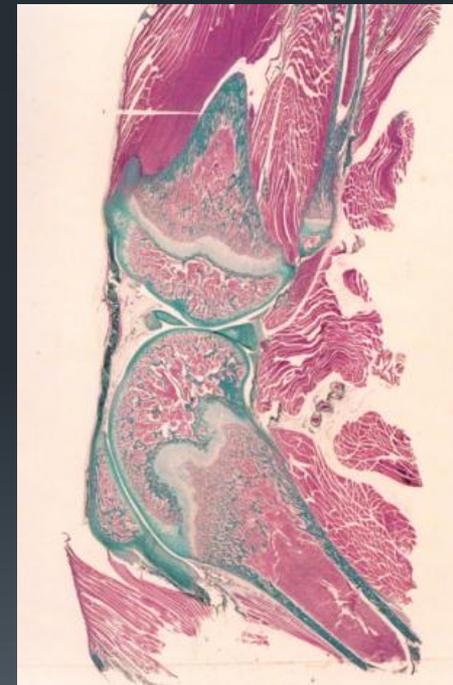
Tecido mineralizado

- **Matriz inorgânica**

- Cálcio
- Fósforo
- Magnésio
- Potássio
- Sódio
- Citrato

Métodos de descalcificação

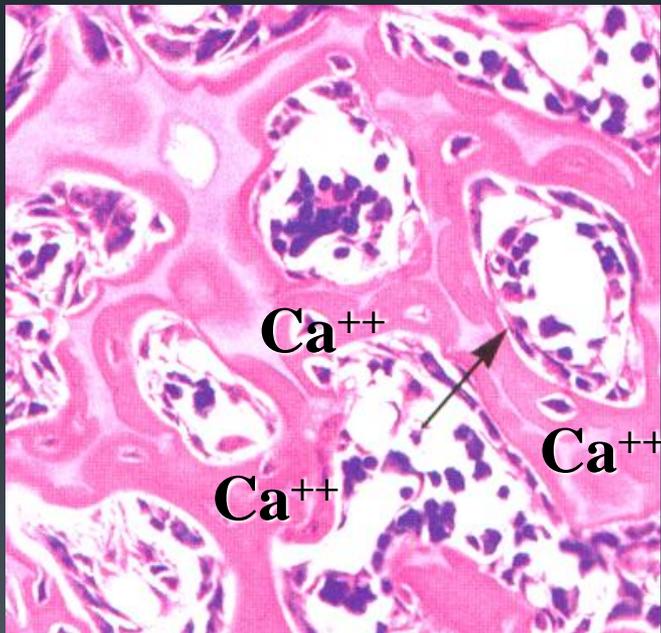
- Soluções ácidas diluídas.
- Soluções quelantes.
- Misturas de soluções ácidas + soluções quelantes.



Mecanismo de ação dos agentes descalcificadores

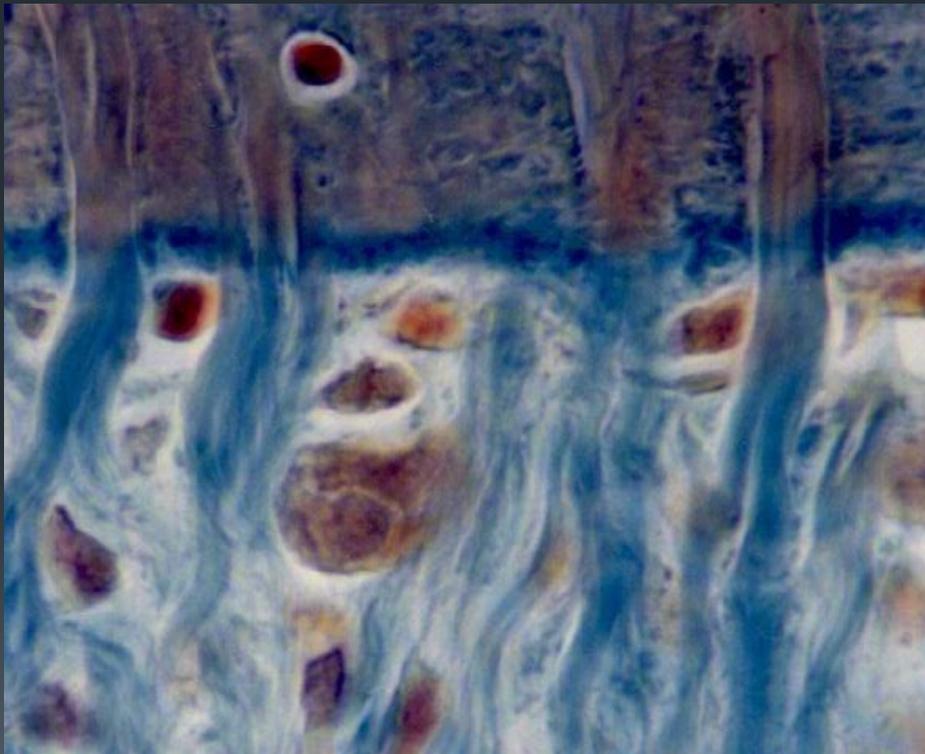
Solução descalcificadora

Ácido (H⁺)



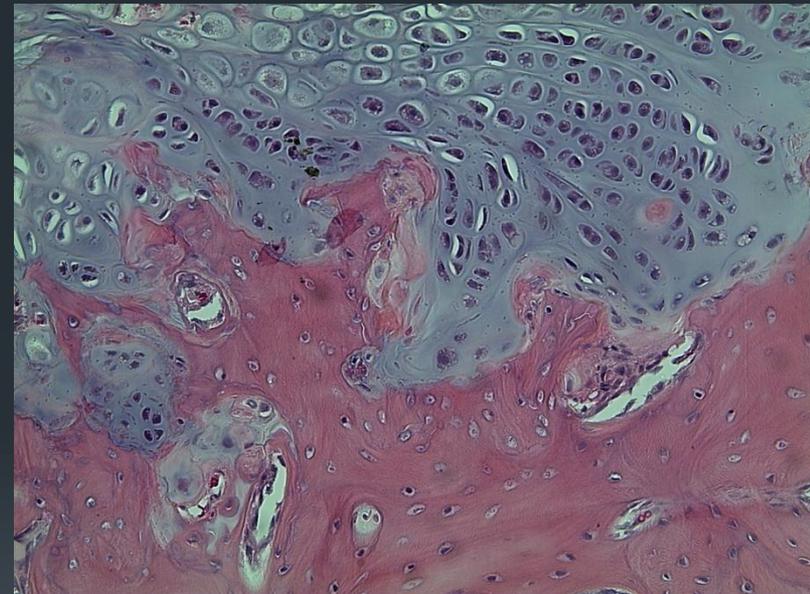
H_3PO_4 (ácido fosfórico) + cloreto de cálcio + água

Descalcificadores mais utilizados

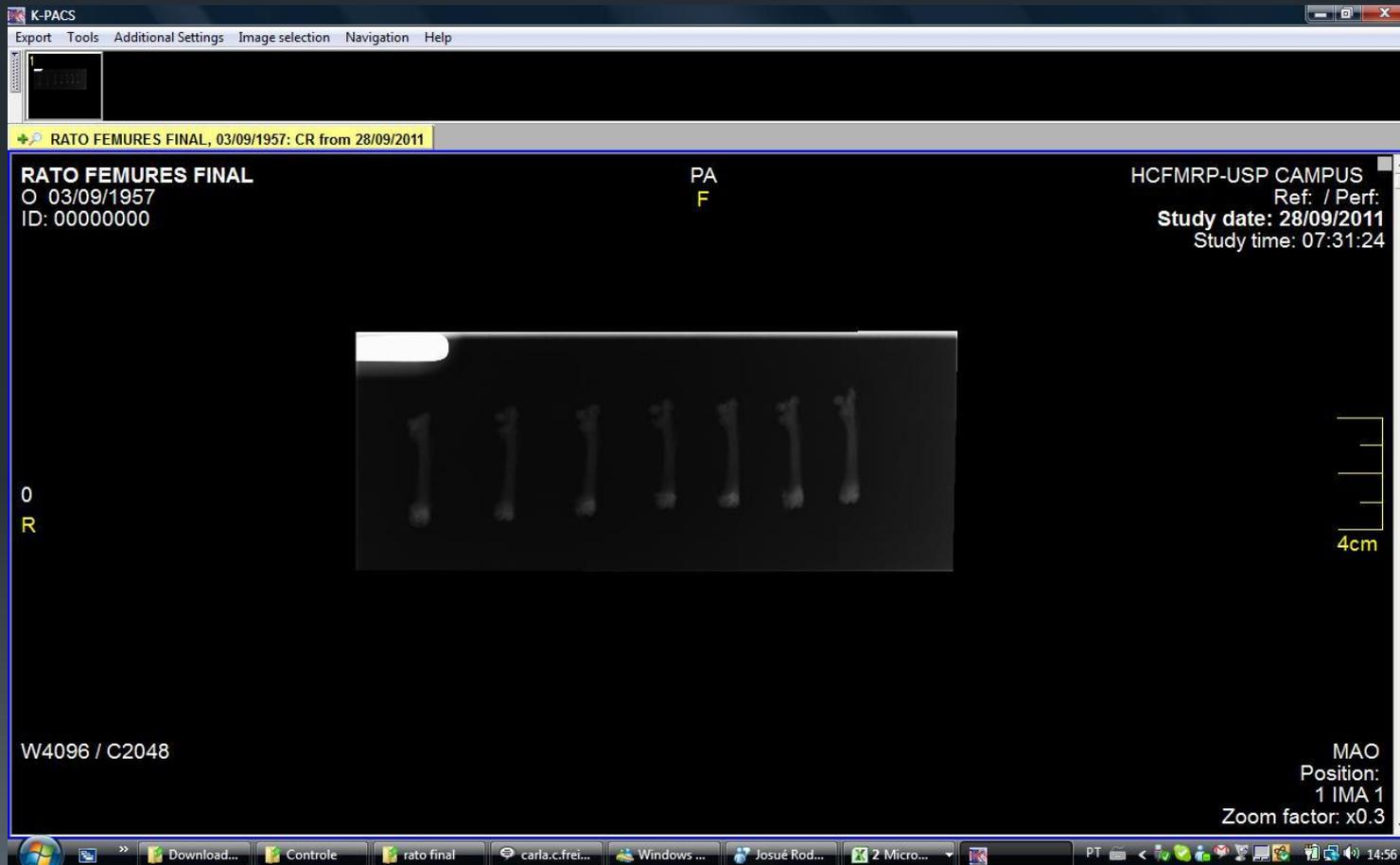


ETDA

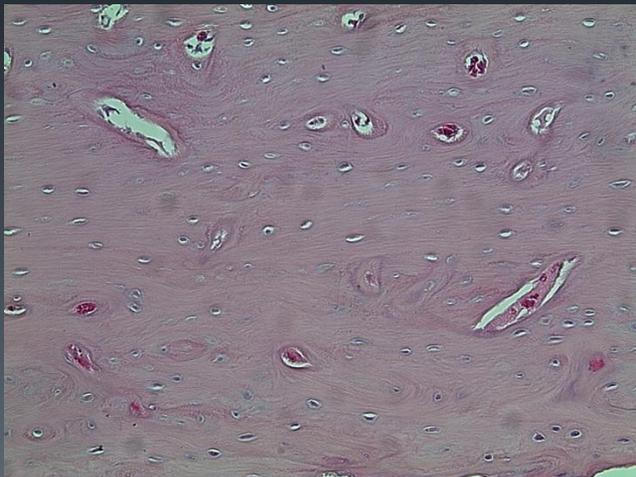
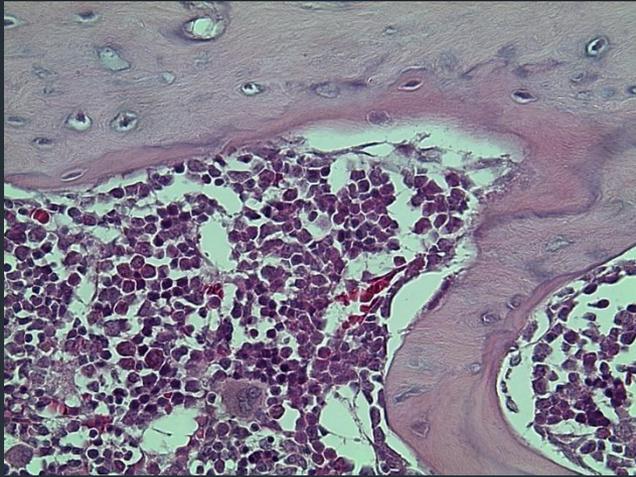
- EDTA. 0,7g
- Tartarato de sódio e potássio. 8g
- Tartarato de sódio. 0.14g
- Hcl. 120ml
- H2O destilada. 900ml



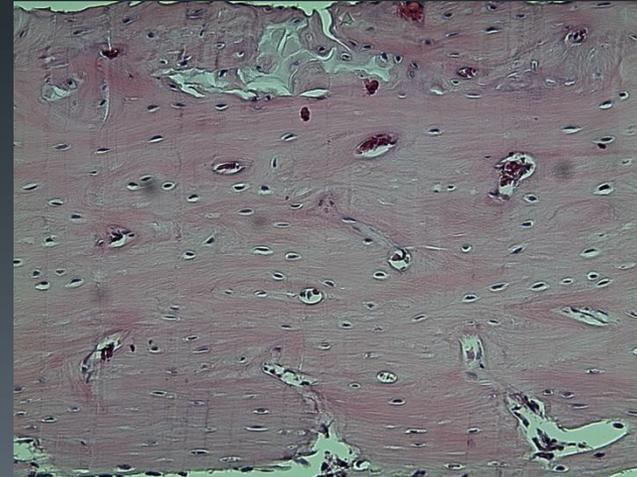
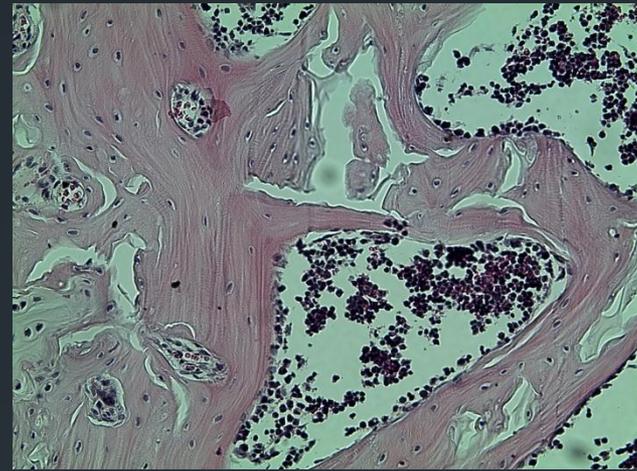
FÊMUR ETDA DESCALCIFICADO EM 24 HORAS



ETDA



EDTA



Cuidados com a descalcificação

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

Tempo para a troca do descalcificador

Table II: Decalcification tissue by EDTA

TIME	EDTA-IMMERSION [Ca ²⁺], ppm	EDTA- MICROWAVE [Ca ²⁺], ppm
6 hours	142.3
2 days	2425.8
3 days	948.8
5 days	5.9	385.7
15 days	5.1	77.3
35 days	2373.3	12.3
65 days	4113.0
110 days	125.5
150 days	37.3

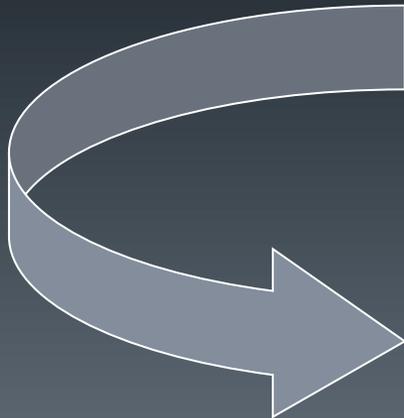
Table I: Decalcification tissue by HNO₃

TIME	HNO ₃ - IMMERSION [Ca ²⁺], ppm	HNO ₃ - MICROWAVE [Ca ²⁺], ppm
6 hours	1242.3
2 days	2425.8
3 days	948.7
5 days	6.5	385.7
15 days	20.3	43.4
35 days	4953.8
65 days	433.1

Como saber o ponto ideal da descalcificação ?

Métodos mais utilizados

- Método radiográfico.
- Flexibilidade do tecido.
- Utilização de alfinete.
- Método quantitativo.



Amônia Concentrada.
Solução Saturada de Oxalato de
Amônia.
Trocar o Descalcificador a cada 24
horas e fazer o teste

Método Quantitativo

- Pegar 5ml de Descalcificador do fundo do Frasco.
- Acrescentar 1 ml de Amônia concentrada (Misturar).
- Acrescentar 0,1ml de Oxalato de amônia.
- Deixar por 10 minutos para verificar a presença de cálcio.
- Se não formar precipitado acrescentar mais 0,1ml , com intervalo de 10 minutos.
- Até no Máximo 0,6 ml .
- Se não formar precipitado o tecido e considerado descalcificado.

FLEXIBILIDADE OSSO

VI CONGRESSO REGIONAL DE
HISTOTECNOLOGIA, RIO DE
JANEIRO FIO CRUZ

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Flexibilidade Dente

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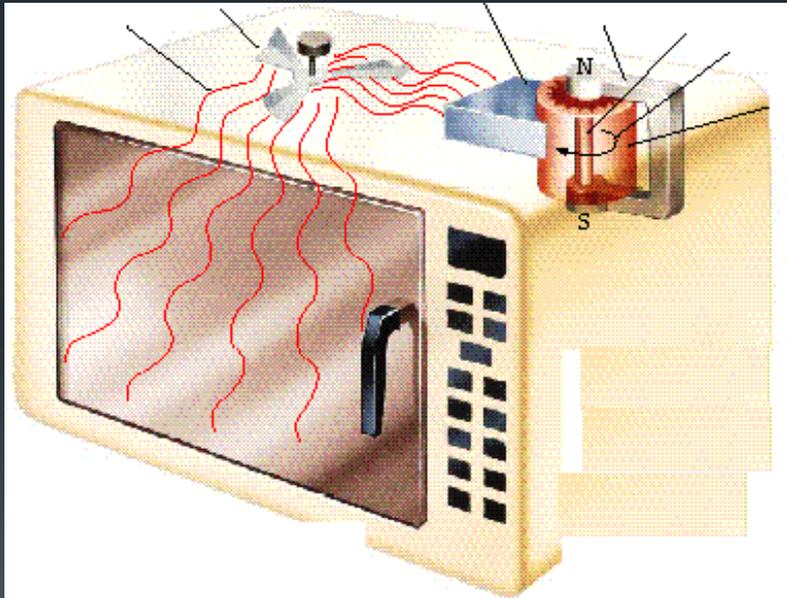
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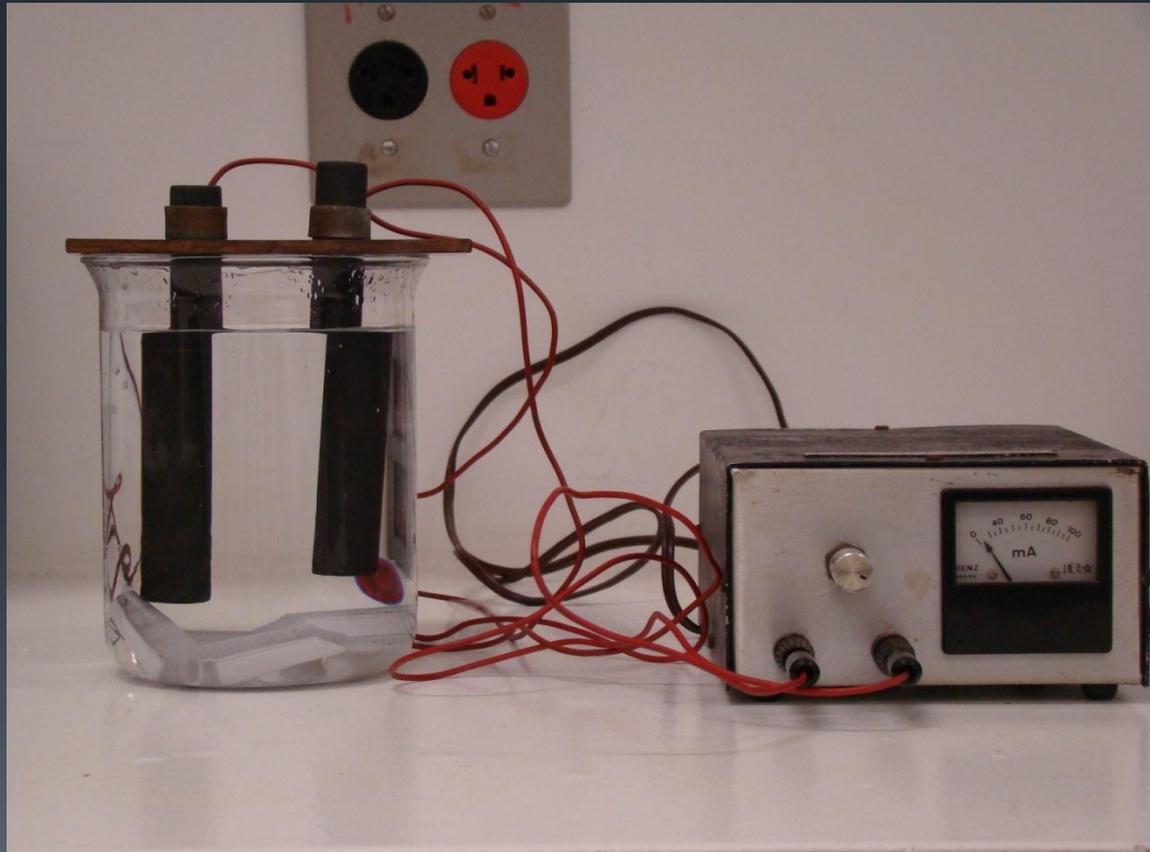
Cuidados depois de descalcificar com EDTA



Métodos para agilizar a descalcificação



Métodos para agilizar a descalcificação

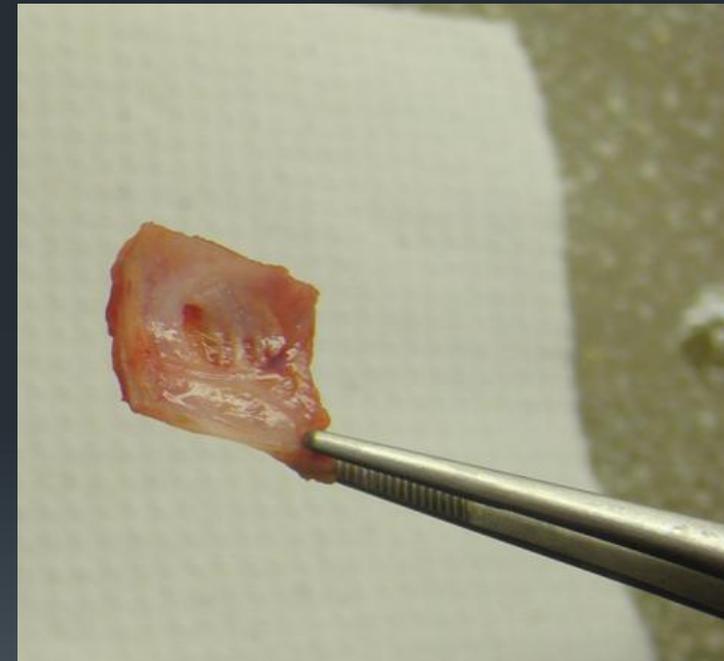
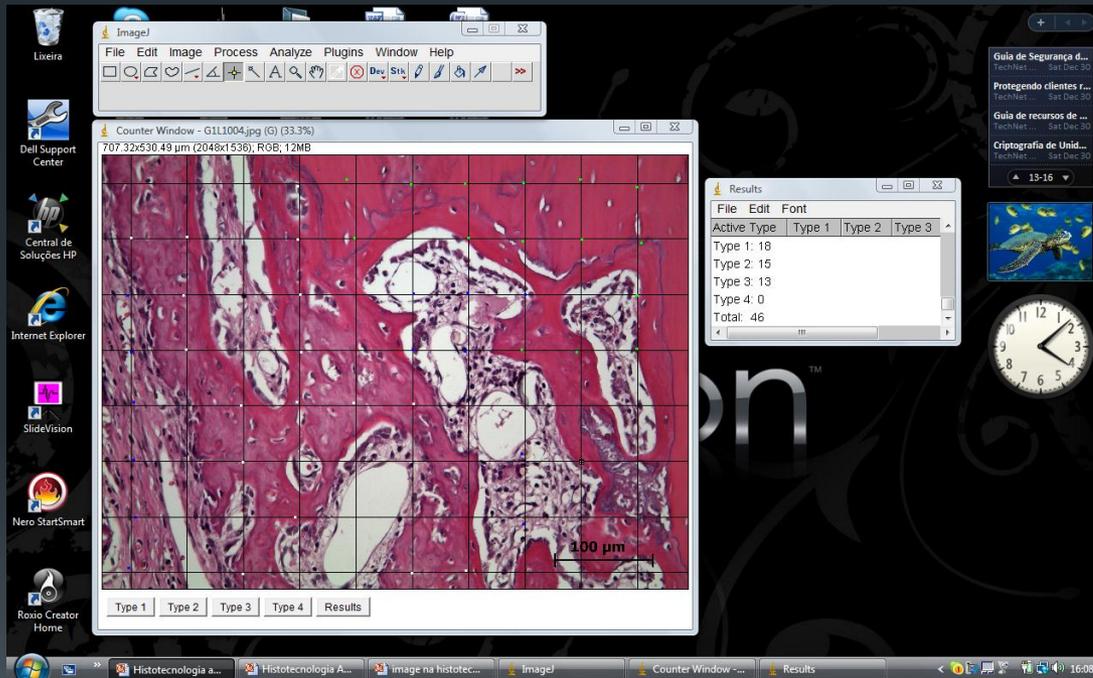


Adesão dos cortes

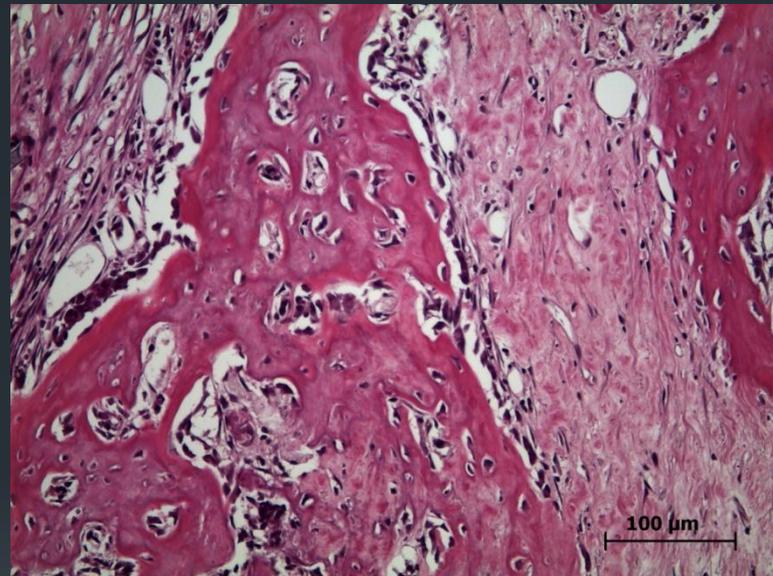
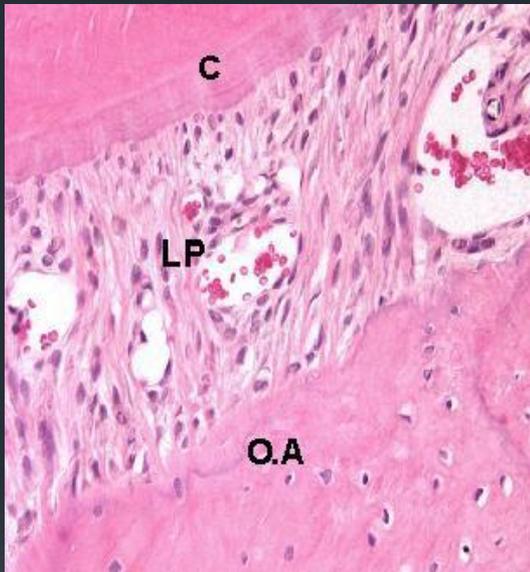


- Ideal é cortes com 5 a 3 μ m.
- Lâminas silanizadas
- Poly-L- lisina.
- Albumina (não)

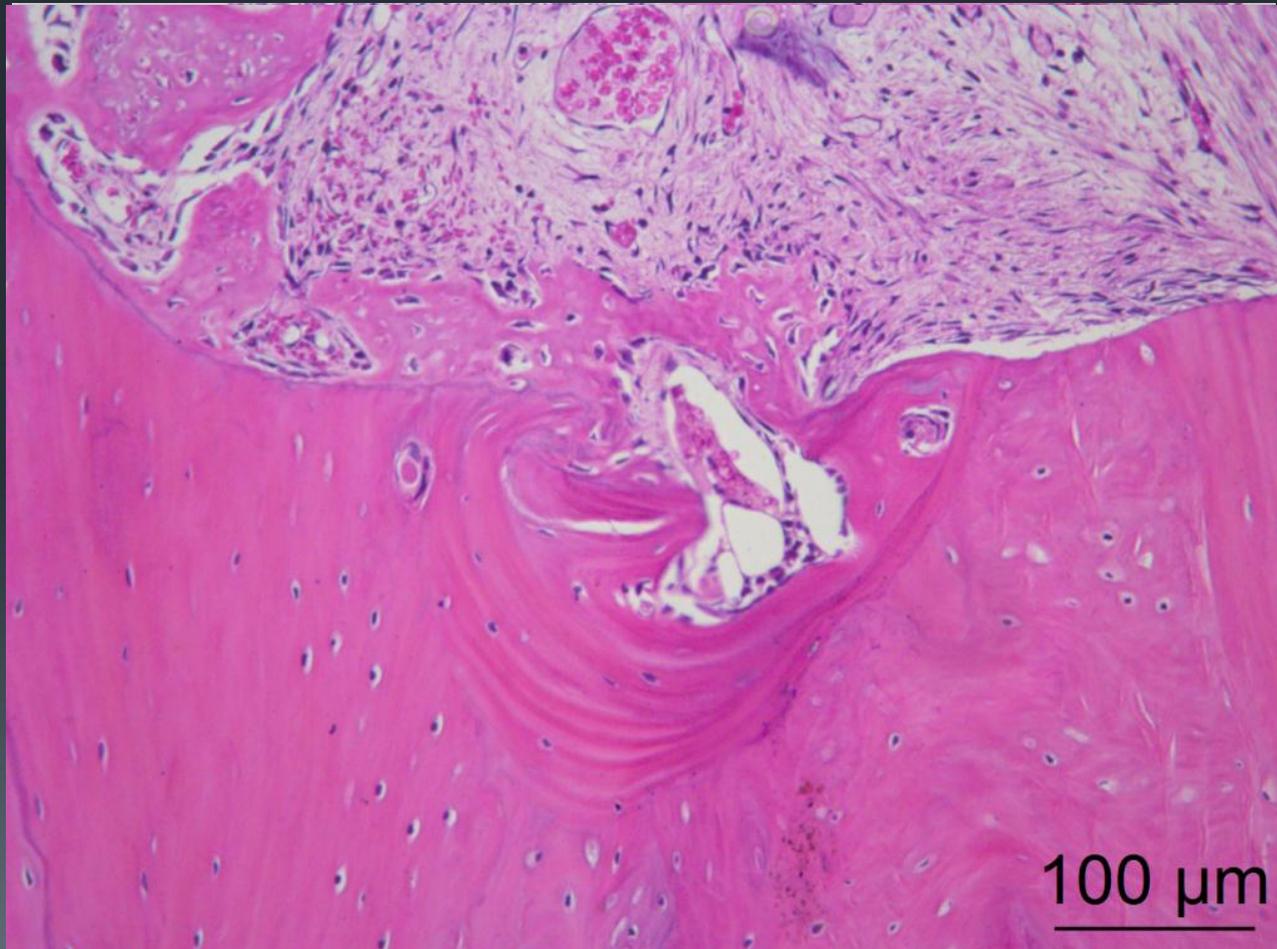
Cortes seriados



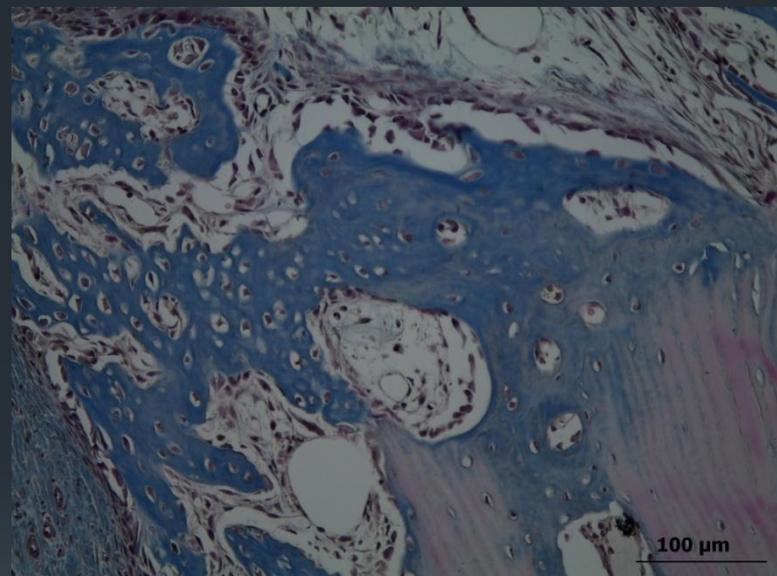
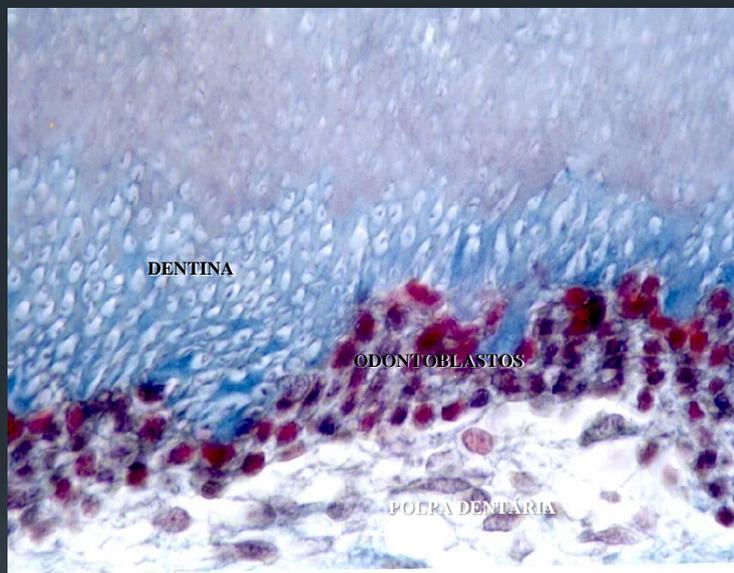
Hematoxilina Eosina



Hematoxilina Eosina



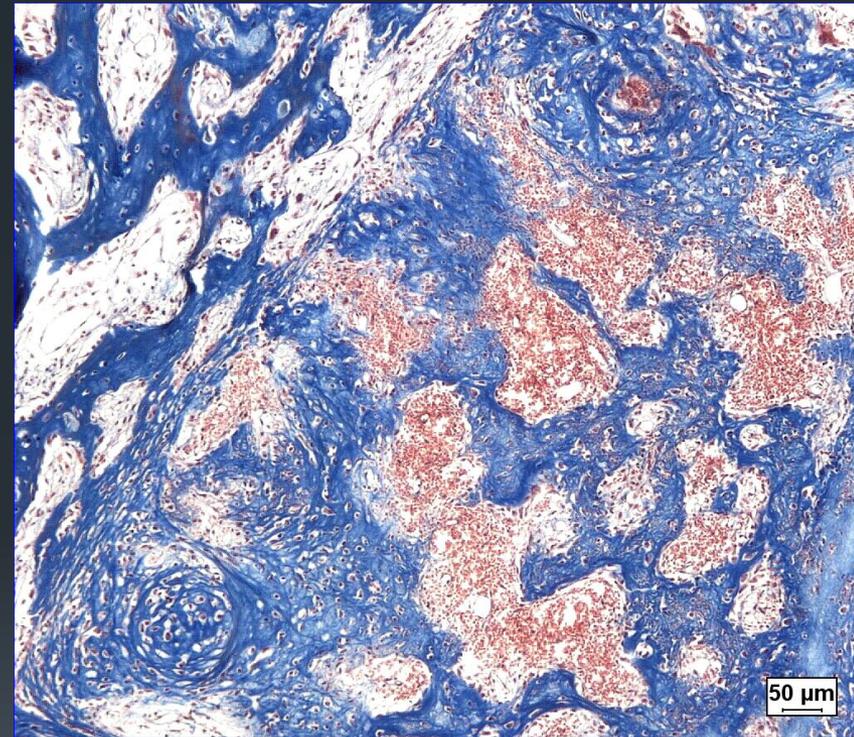
Tricômio Masson



Sequência do processo de neoformação óssea

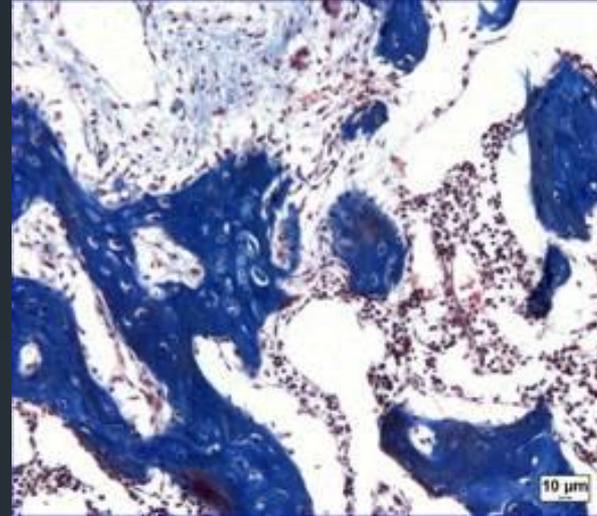
Fase angiogênica inicial:
Proliferação dos vasos
sanguíneos, com formação
do coágulo.

**Fase de formação de osso
novo:** Tecido de granulação,
tecido conjuntivo e tecido
ósseo imaturo.

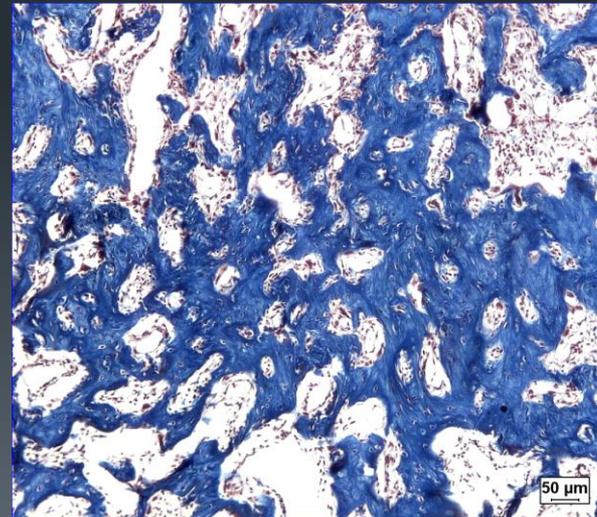


Sequência do processo de neoformação óssea

Fase de crescimento:
Formação de tecido ósseo
imaturo e posterior
mineralização.



Fase de reorganização:
Trabéculas primárias do
osso remodelam-se para
formar uma camada
esponjosa mais espessa e
um tecido ósseo mais
denso.



Historesina Leica



Historesina



Adaptações no protocolo de Imunohistoquímica

- Diminuição do número de lavagens.
- Bloqueio de peroxidase endógena.



Protocolo tecido ósseo

- Diluição do anticorpo primário em BSA.
- Incubação do anticorpo por 2 horas.
- Uma lavagem de 5 minutos em PBS.
- Encubar com anticorpo secundário “conjugado” por 40 minutos.
- Uma lavagem de 5 minutos.
- Aplicação do DAB (Cromógeno) por 5 minutos.
- Uma lavagem em PBS por 5 minutos.
- Passar um minutos na hematoxilina, desidratar e montar com lamínula.



Anticorpos

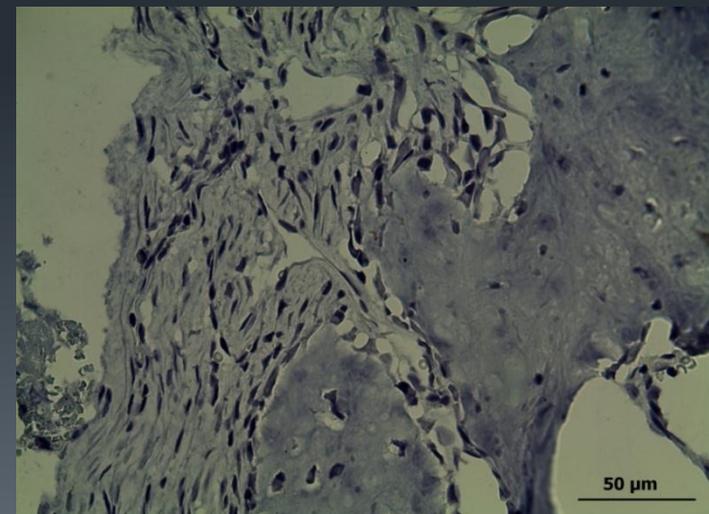
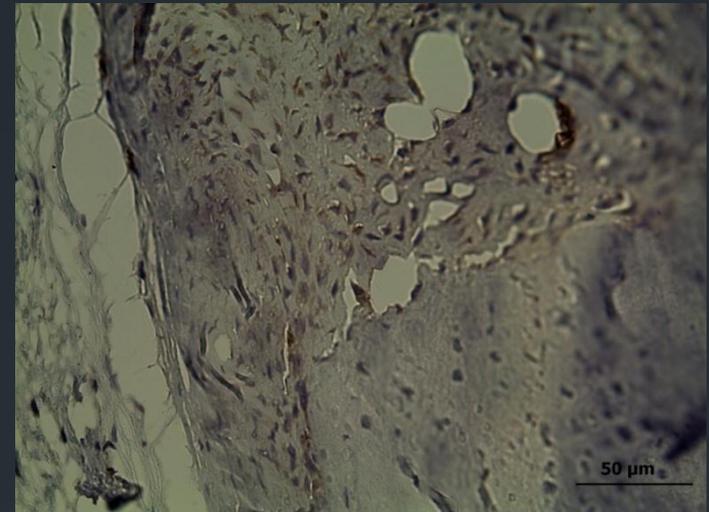
- MMP-9 Ab 19016 (CHEMICON)
- CD 34 Sc 9095 (SANTA CRUZ)
- CD 31 Ab 64543 (ABCAM)
- FGF BASIC Ab 16828 (ABCAM)
- VEGF R2 Ab2349 (ABCAM)
- VEGF Ab44714 (ABCAM)
- OSTEOCALCINA Sc 30044 (SANTA CRUZ)
- SIALOPROTEINA Ab 1851 (CHEMICON)

VEGF R2

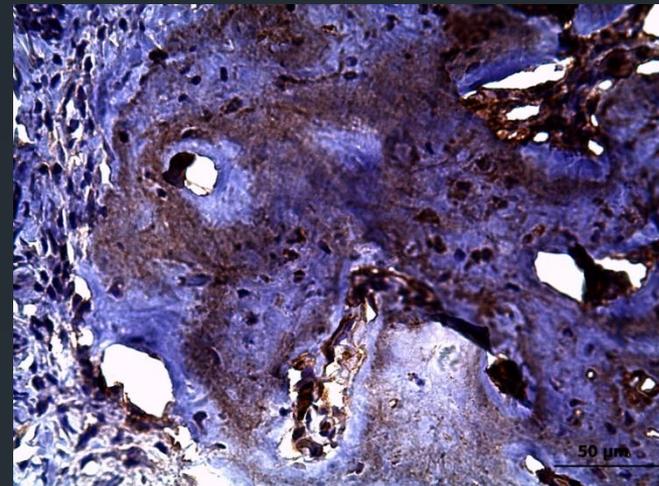
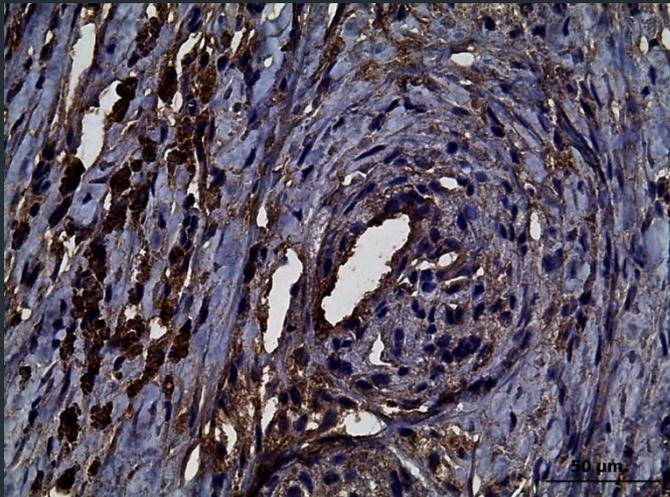
Calvária VEGFR 2 40X.

Diluição 1/50 micro-litros

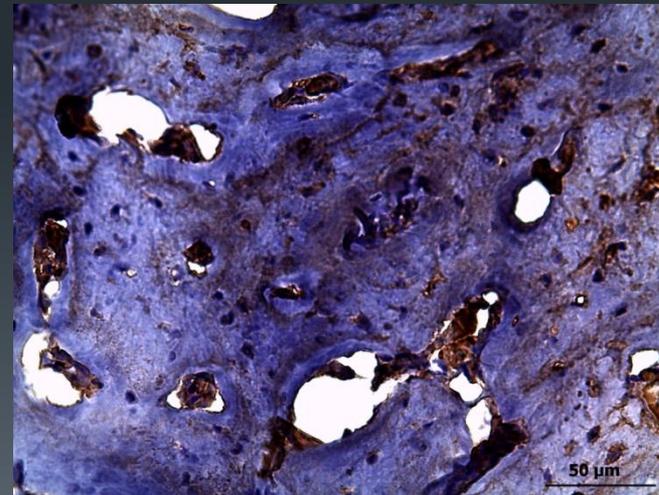
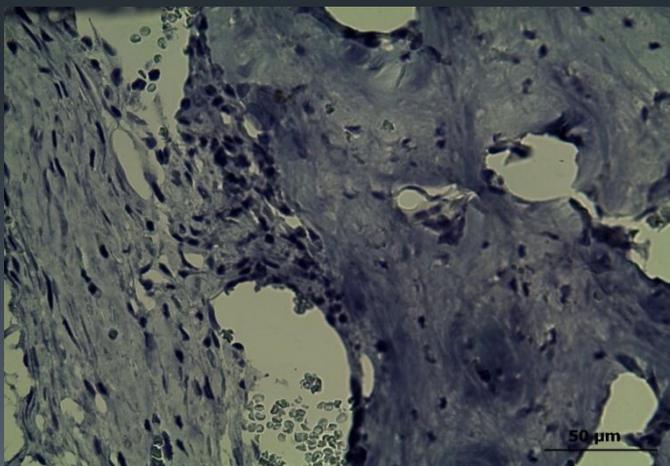
Calvária VEGFR 2 CONTROLE
NEGATIVO 40X.



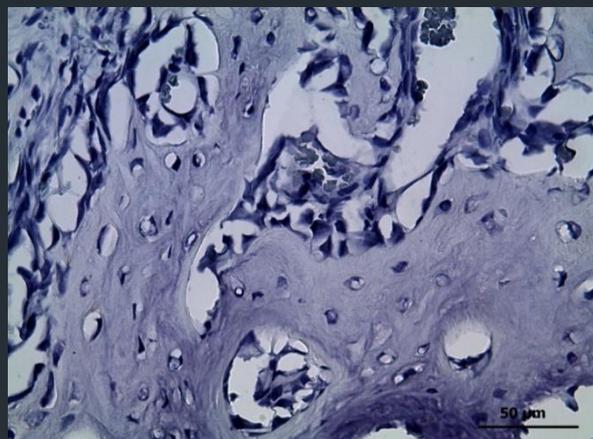
Osteocalcina



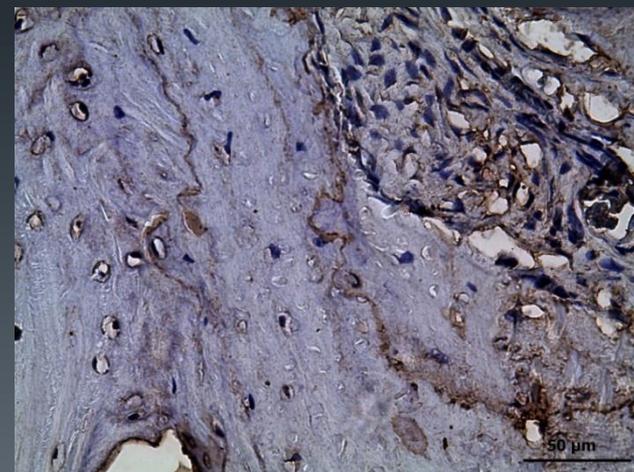
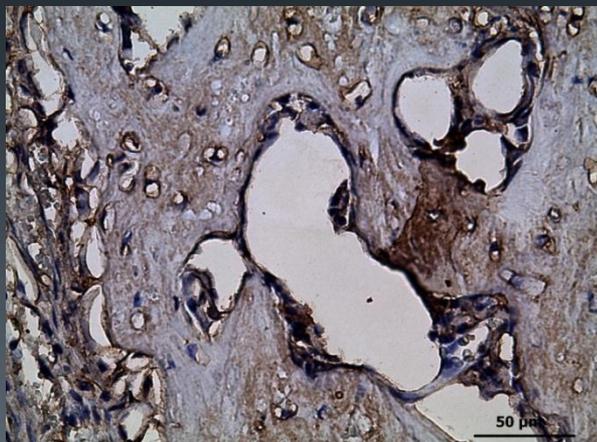
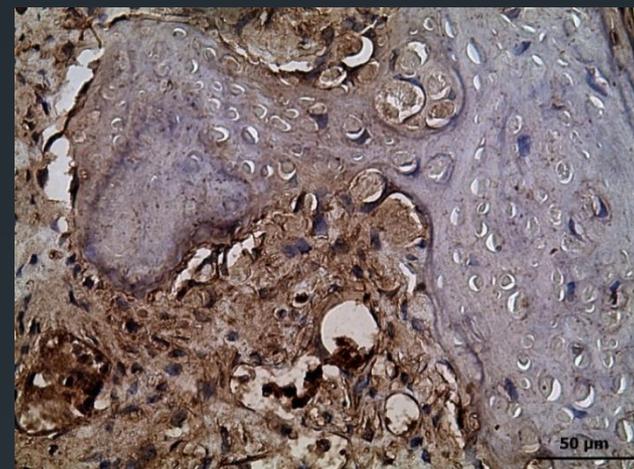
1/50



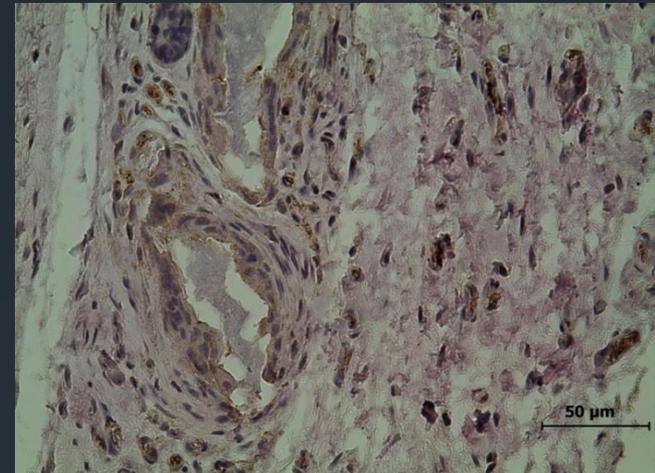
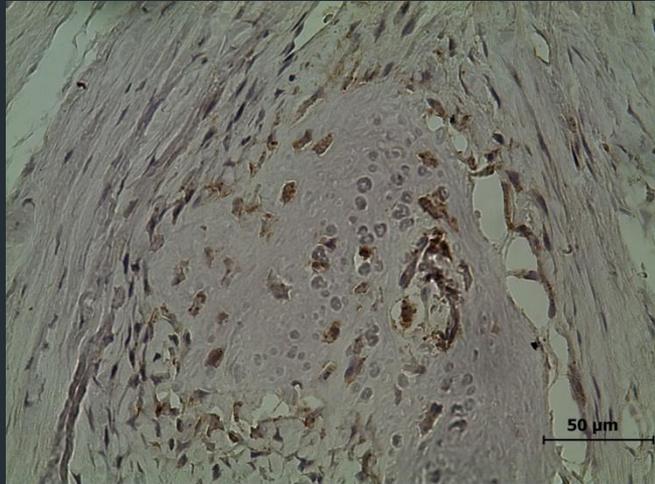
Sialoproteína II



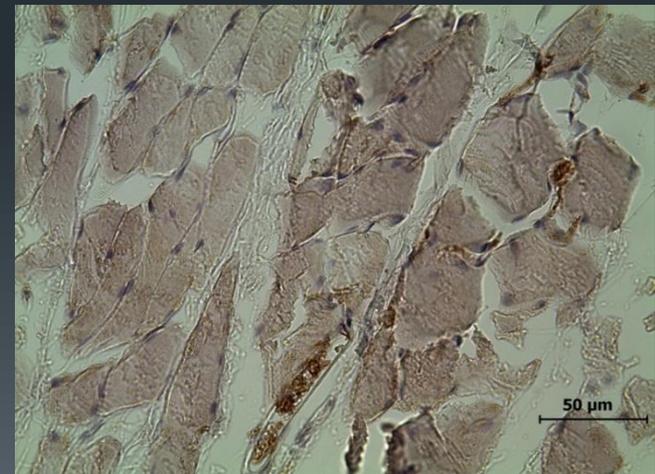
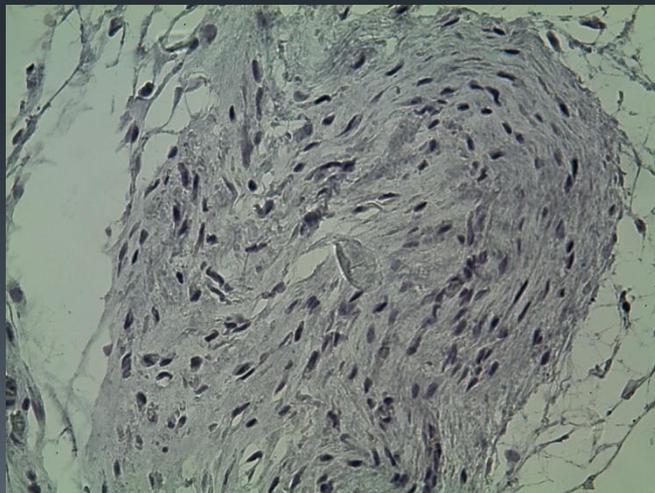
1/ 2.500



TGF Beta



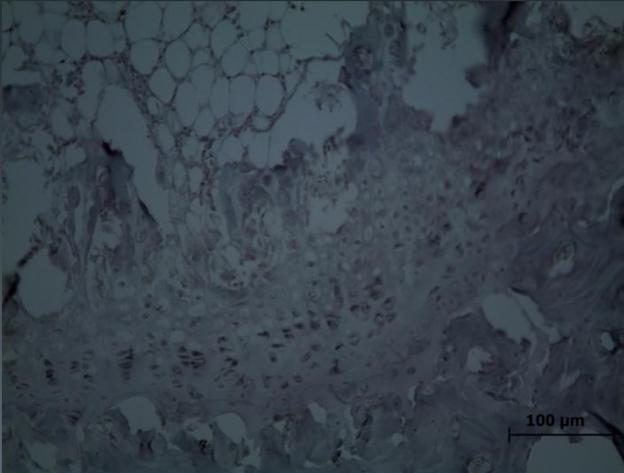
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MMP-9



1 / 50





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2 Collagen fibers evaluation after rhBMP-2 insertion in critical-sized defects

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ABSTRACT

The objective of this investigation was to assess the quantity of collagen fibers with different dosages of recombinant human bone morphogenetic protein, type 2 (rhBMP-2) associated with two different carriers, monolaminar and polyaxamer gels, in critical bone defects created in the calvaria of Wistar rats. Forty male adult Wistar rats were divided into eight groups of 5 animals each: group I: critical bone defect with application of 1 µg of rhBMP-2 combined with monolaminar gel; group II: 3 µg of rhBMP-2 combined with monolaminar gel; group III: 7 µg of rhBMP-2 combined with monolaminar gel; group IV: 1 µg of rhBMP-2 combined with polyaxamer gel; group V: 3 µg of rhBMP-2 combined with polyaxamer gel; group VI: 7 µg of rhBMP-2 combined with polyaxamer gel; group VII: monolaminar gel only and group VIII: polyaxamer gel only. A critical-sized defect of 6 mm diameter was produced in the left parietal bone using a surgical round bur and a high-speed micromotor. The bone defects were filled according to the group that animals belonged and after two weeks the rats were perfused and their calvarial bones were removed for histological processing, and collagen fibers quantification. Differences among the eight groups were statistically analyzed by Anova and Bonferroni test ($p < 0.05$). The results did not show statistical difference between the groups, in exception, between the comparisons II and III. According to the experimental methodology used in this research, it was observed, in a general way, a qualitative inverse relationship (plot) when collagen fibers presence and rhBMP-2 quantity inserted in the critical bone defect, associated or not to a material carrier.

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1. Introduction

One of the primary factors for the development of bone tissue is the production of a number of growth factors by osteoblasts and by their precursors (pre-osteoblasts), or both of them. The osteoblasts secrete several members of the superfamily of bone morphogenetic proteins (BMP), including morphogenetic protein types 2 and 7, which have an important role in the bone repair, whose mechanism is named as osteoinduction.

Nowadays, the BMP carriers are widely tested and they must have some properties such as: not to induce inflammatory or immune reactions, be absorbed during the bone healing process, be free of toxic residues, and supply a sustainable delivery system of

the protein. In addition, they should be easily and cost-effectively manufactured for large-scale production, possible to be sterilized and also present appropriate storage conditions and stability (Seeherman and Wozney, 2005).

The collagen is the most representative class of insoluble fibrous protein found in extracellular matrix and connective tissues, and it is responsible for the initial structural conformation of the tissues. Currently, the literature indicates around 18 types of collagens, with their subunits. The collagen types I, II and III are the most abundant in the human body, and as a consequence of this fact, they are the most studied in the literature. The collagen type I is present in skin, tendon and bone, the type II in cartilage and vitreous humor, and type III in skin and muscles.

Structurally, the collagen molecule is represented by a small stick and formed by interlacing in triple helix of three polypeptide chains named alpha chains. Added to this description, the conformation of this protein structure justifies the physical and biological properties of the collagen, which are generally able to provide rigidity, strength and stability.

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Obrigado !

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