Erasmus+
Jean Monnet Modules

A NEW HETEROLOGOUS FIBRIN SEALANT IN REGENERATIVE BIOMEDICINE

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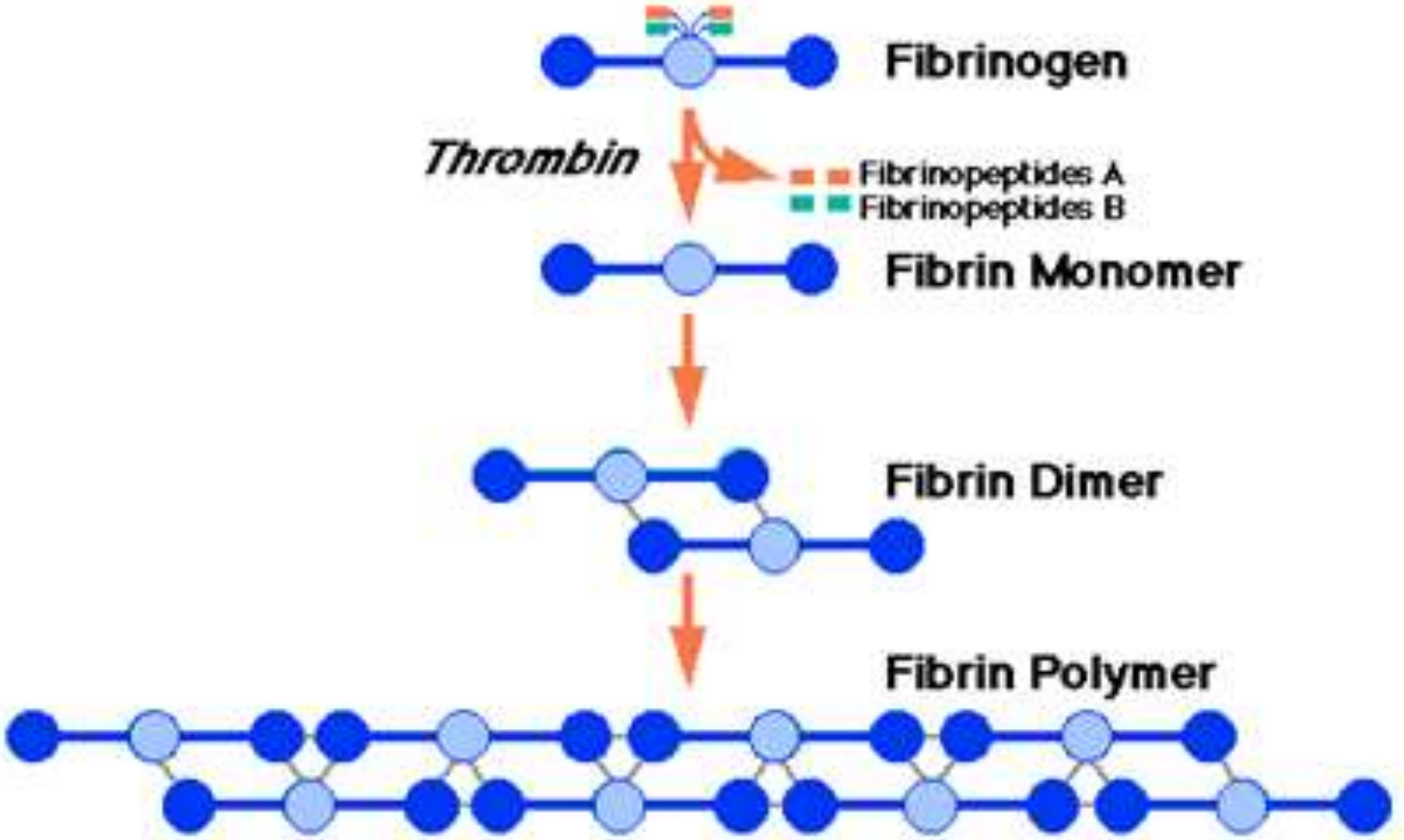
MED Medical Institute Sumy State University

https://www.google.com.ua/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&cad=rja&uact=8&ved=0ahUKEwj17s2Ag5bYAhVFPRQKHQHxAgMQjRwIBw&url=https%3A%2F%2Fwww.worldatlas.com%2Fsa%2Fbr%2Fsp%2Fwhere-is-botucatu.html&psig=AOvVaw2RIvtIh43FgjsaX4f2QJ5Z&ust=1513771041414371

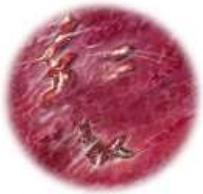


https://i.pinimg.com/originals/81/00/0f/81000ffc38b5909d898bf934c7f6b0f5.gif

EVICEL™ Fibrin Sealant (Human)

TachoSil®
Fibrin Sealant Patch

- Forms an elastic clot that anchors firmly to the bleeding site[2]
- Sprays or drips for broad or targeted application[1]
- 5 mL provides 100 cm² coverage area[1]
- Provides clear visibility of the bleeding site so you can quickly assess hemostasis[2]
- EVICEL™ maintains clot stability over time comparable to other fibrin sealants, without the need for an antifibrin



RAPLIXA®
(Fibrin sealant (human))
for topical use

www.tachosilus.com/
hemostatsolutions.com/raplixa/
www.slideshare.net/79536/plastics-8767130
www.vivostat.com/products/vivostat-fibrin-sealant
www.ethicon.com/healthcare-professionals/products/biosurgery/evarrest-fibrin-sealant-patch
www.exportersindia.com/dolphin-surat/reliseal-fibrin-sealant-kit-ip-surat-india-1470373.htm
www.dotmed.com/listing/disposables-general/ethicon/3905/evicel-fibrin-sealant/2365796

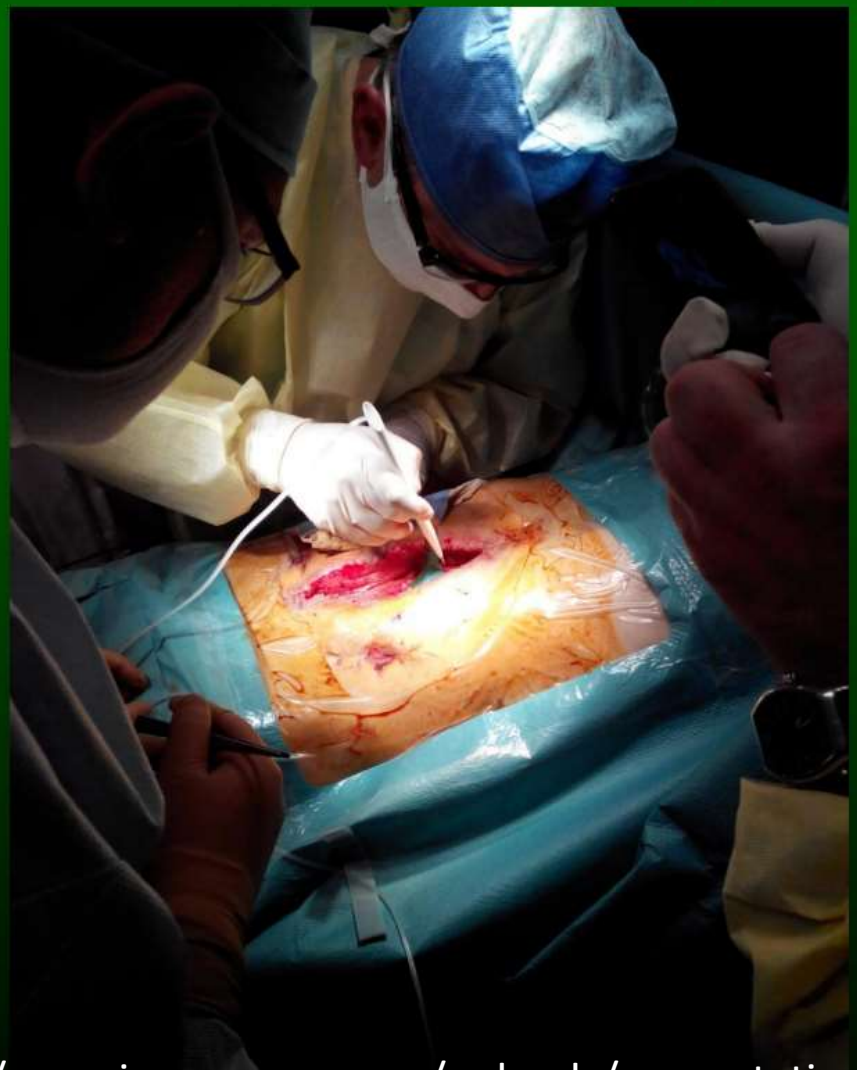
Фібриновий клей як альтернатива хірургічним швах

Фахівці з пластичної хірургії придумали, яким чином можна скоротити післяопераційний період до одного тижня замість шести: вони замінили шви на високотехнологічний клей. Фібриновий клей прискорює процес загоєння ран і скорочує рубці. Часто батьки малюків застосовують для загоєння ранок біологічний клей, який має бактерицидну і загоює діями. Не так давно аналогічну ідею почали використовувати в косметичній хірургії століття, як альтернативу хірургічним швах. “Фібриновий клей почав широко розповсюджуватися в нейрохірургії. Але це досить дорогий продукт, і тому його застосування має бути обґрунтоване”, - каже лицьовій пластичний хірург Джуліан де Сільва. "Після п'ятирічного дослідження на цю тему, проаналізувавши отримані дані, я зробив висновок, що розчин не тільки за короткий час склеює тканини, але і скорочує післяопераційний період. Пацієнти виписувалися всього через тиждень після операції на відміну від шести тижнів при накладенні швів”, - ділиться пластичний хірург.

http://chitay.net/neopoznanaia-nauka/ua-news-36581.html

Vivostat

(аутогенно збагачений тромбоцитами фібрин та фібриновий клей)



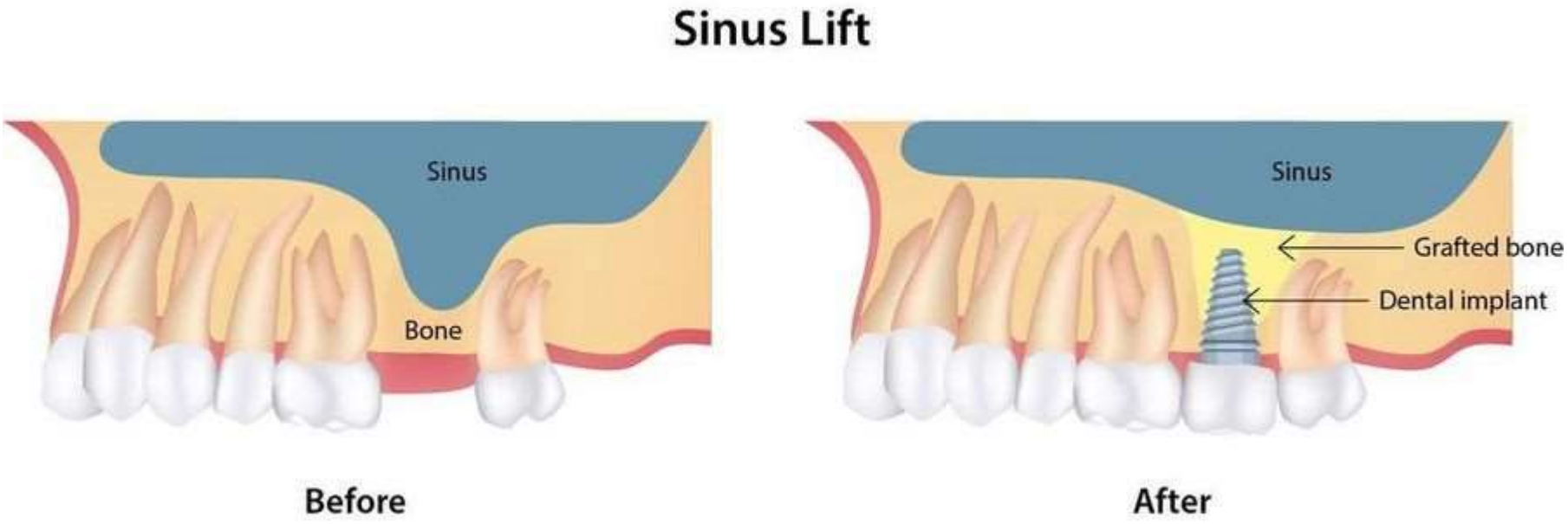
https://www.isurgery.com.ua/uploads/presentations/shurdak.pdf

Vivostat PRF® in burns

For decades fibrin sealant has been used for burns as a scaffold for re-epithelialization. Vivostat PRF® provides this scaffold and combines it with a high concentration of platelets relevant for tissue regeneration. Moreover, it offers the surgeon the opportunity to co-deliver skin cells/stem cells with the Vivostat PRF® solution.

The high concentration of fibrin found in Vivostat PRF®, furthermore, acts as a glue enabling the surgeon to use Vivostat PRF® for graft fixations. Using Vivostat PRF® to fixate the graft allows the surgeon to use less staples or none at all depending on the location of the burn. The fibrin also acts as a haemostatic reducing the risk of haematoma formation, which may cause graft loss. Any remaining Vivostat PRF® can be applied to the graft harvest site to speed up tissue regeneration and reduce pain for the patient.

https://www.vivostat.com/medical-professionals/burns-surgery



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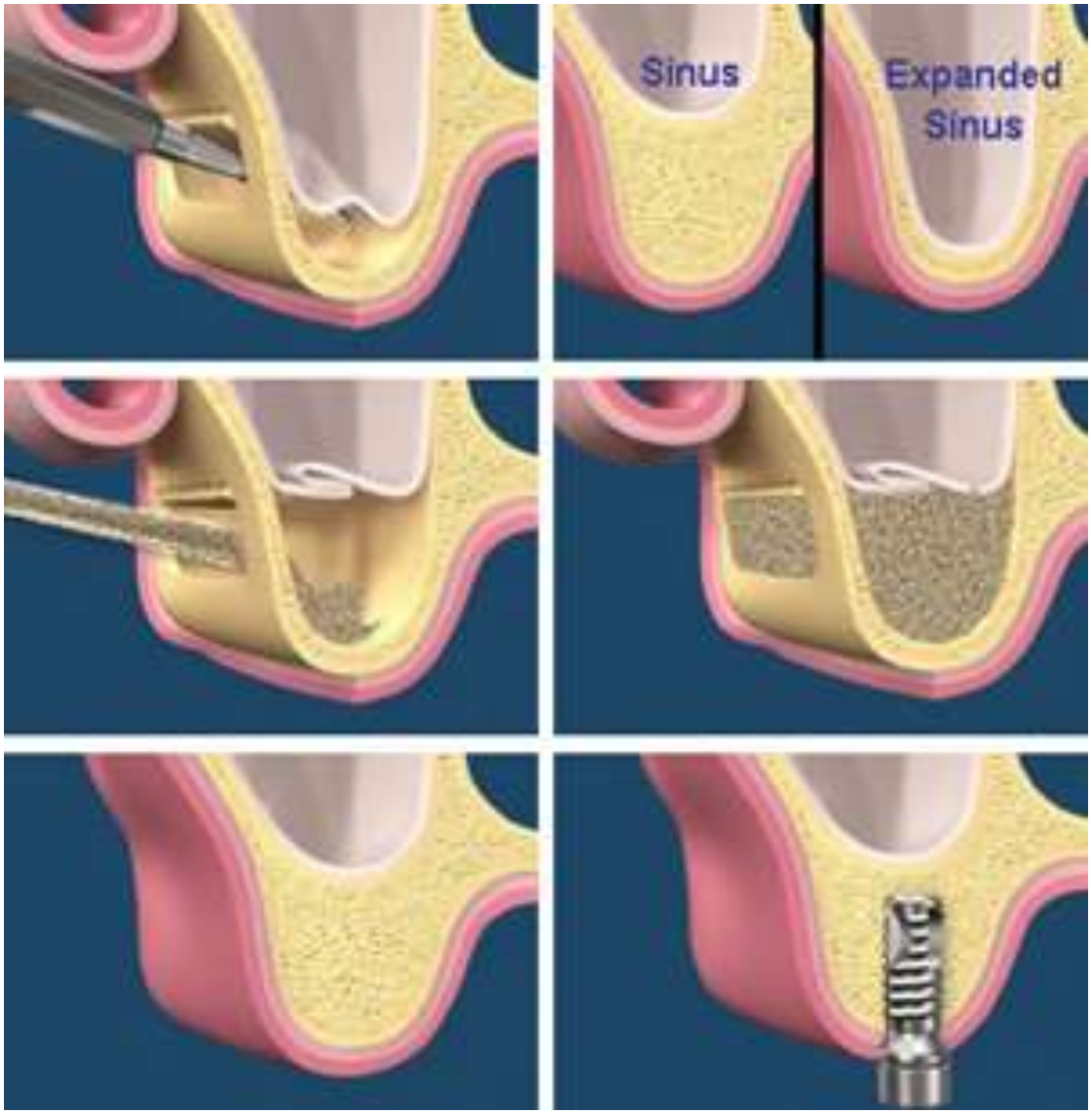

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http://enfoquetriangulo.com/wp-content/uploads/2018/08/Selante-de-Fibrina-CEVAP-Cascavel-Enfoque-Tri%C3%A2ngulo-Jornalista-Eder-Moreira-Capin%C3%B3polis-Uberl%C3%A2ndia-Ituiutaba-Jornalista-Gustavo-Maximiano-Cleber-Camilo-DDD34.jpg


Selante de Fibrina
Estudo Selante: uso exclusivo em ensaio clínico
Uso tópico
Manter armazenado entre -18 e -22°C
Utilizar em temperatura ambiente (15 a 30°C)
Contém 3 frascos - 1 dose
CEVAP
unesp

Ferreira, R. S., Jr., et al. (2017). "Heterologous fibrin sealant derived from snake venom: from bench to bedside - an overview." J Venom Anim Toxins Incl Trop Dis **23: 21.**

Fibrin Sealant - Selante de Fibrina.mp4

clicrdc.com.br/wp-content/uploads/2018/04/pinhao.jpg

Fibrin Sealant

- Surgeries
- Wound healing
- 3D matrix for cell cultures
- Biomatrix for 3-D bioprinting

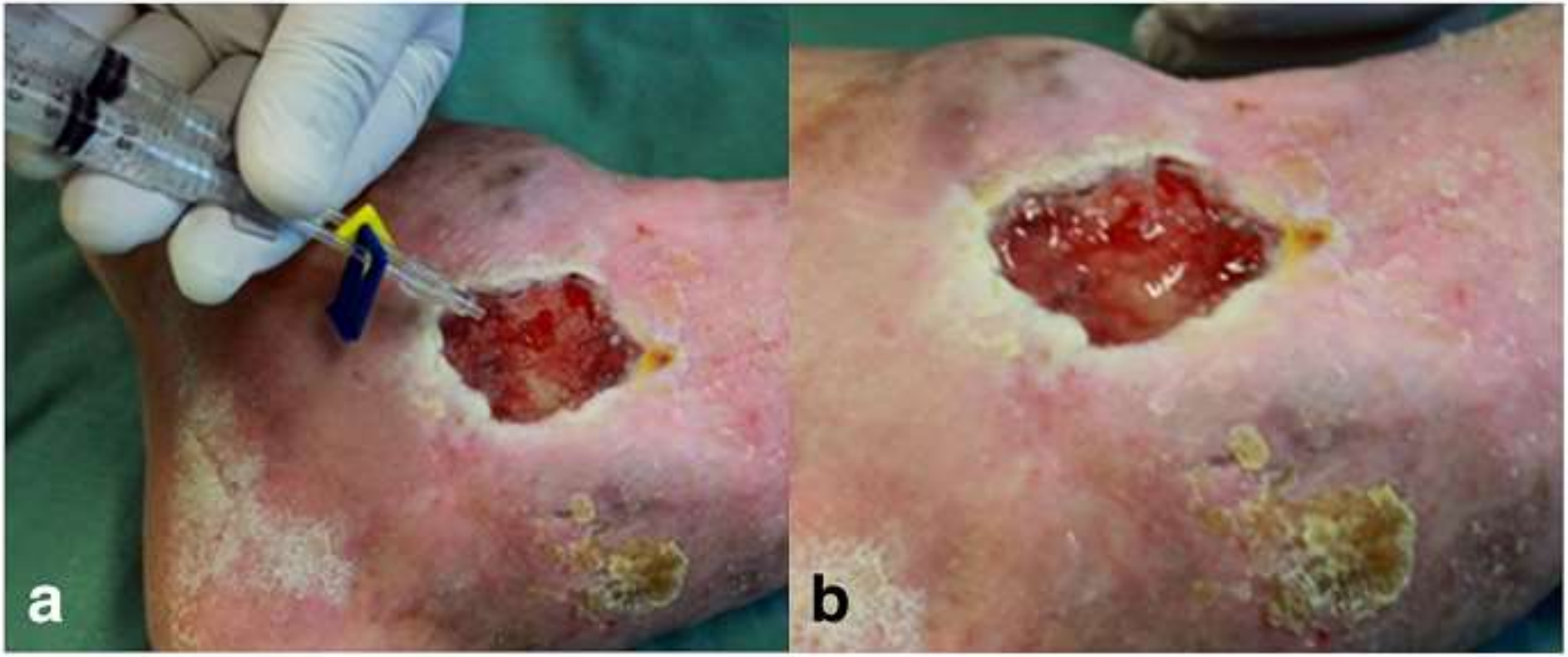


Fig. 12 a Application of the product utilizing a double-outlet syringe with mixer at its end. b Polymerized product covering an ulcer

Ferreira, R. S., Jr., et al. (2017). "Heterologous fibrin sealant derived from snake venom: from bench to bedside - an overview." J Venom Anim Toxins Incl Trop Dis **23: 21.**

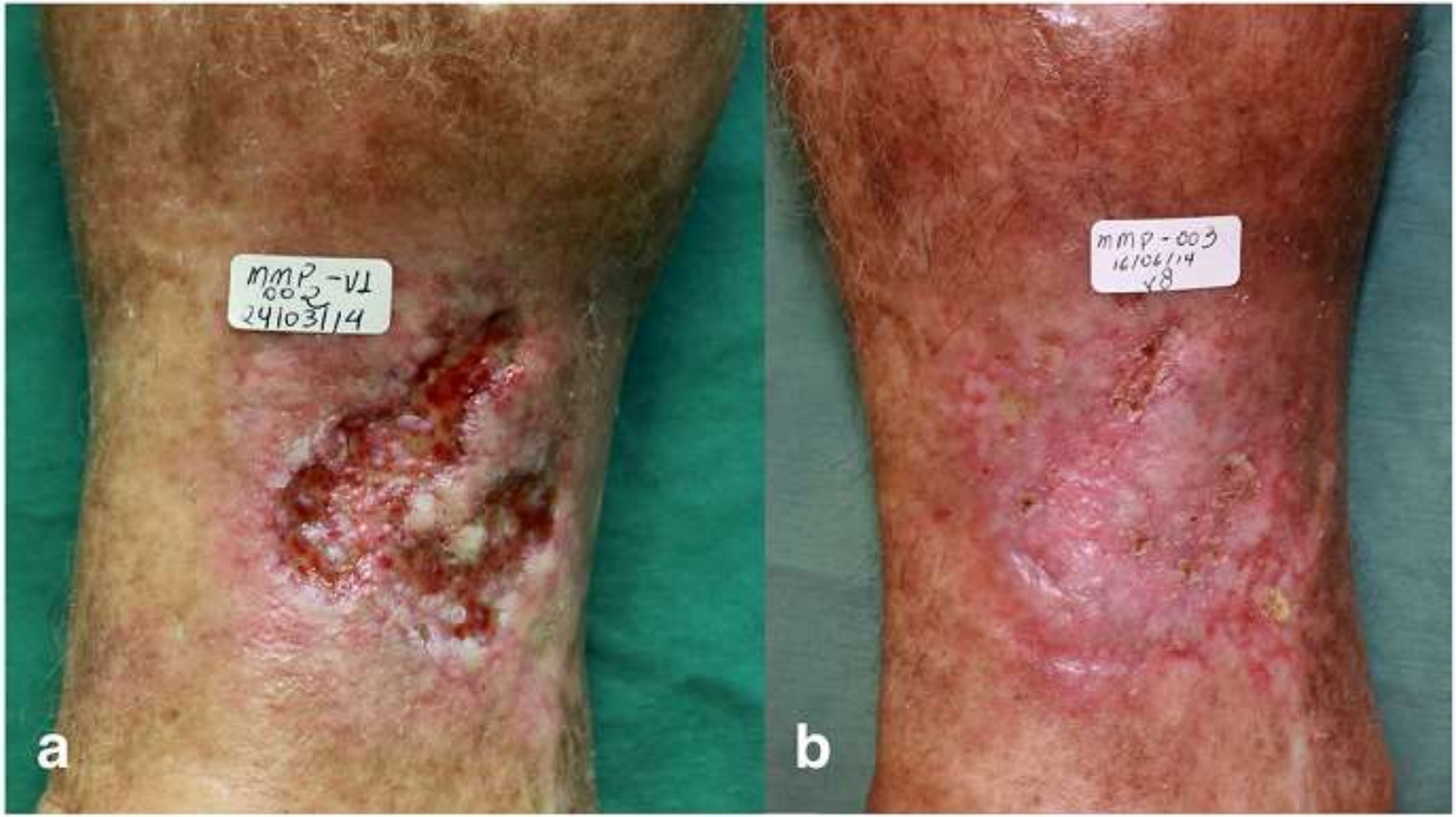


Fig. 13 A 70 year-old female had an ulcer for two years. a Visit 0 – area of the ulcer was 17.1 cm2. b Visit 6 – wound healed

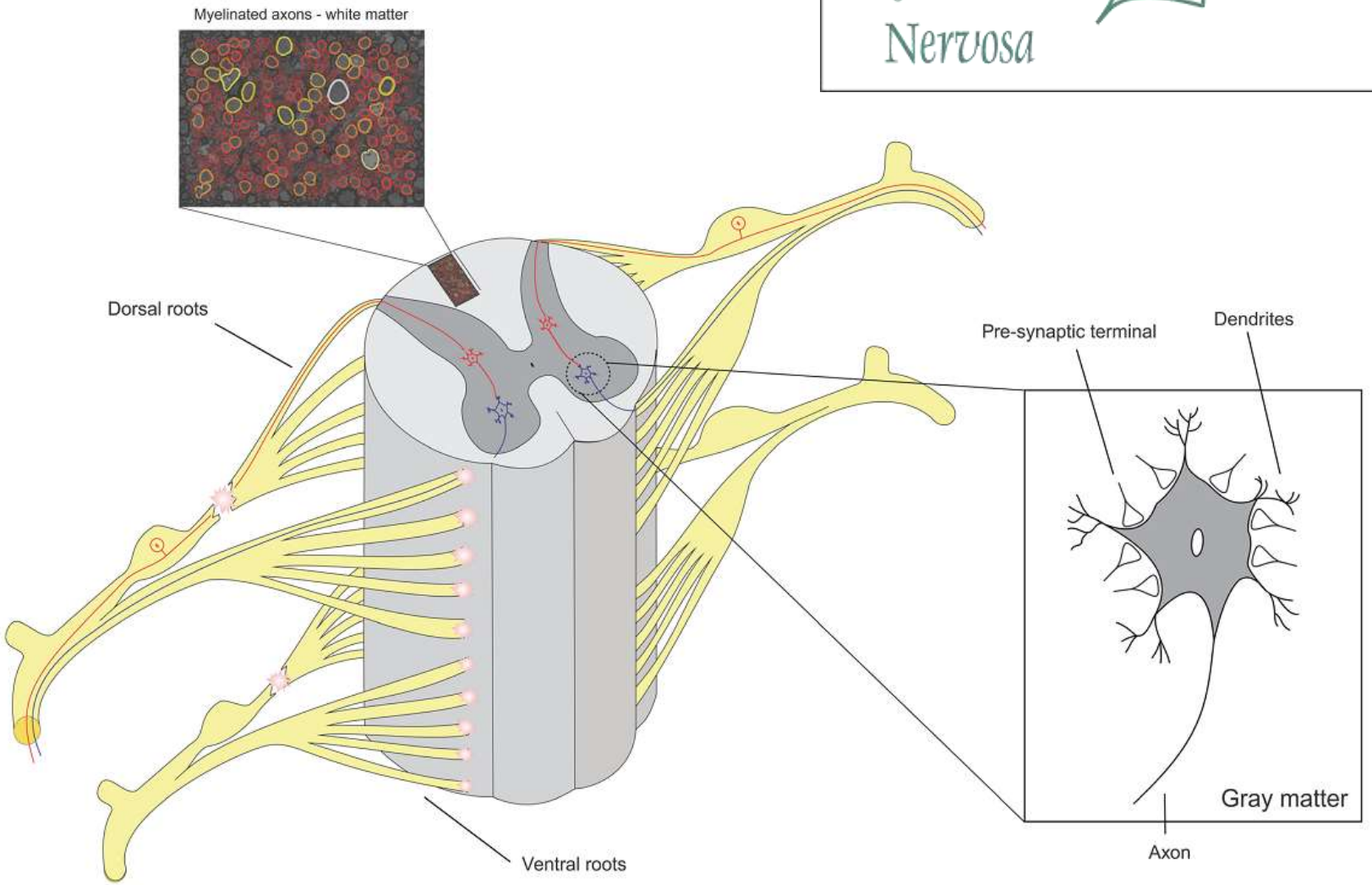
Ferreira, R. S., Jr., et al. (2017). "Heterologous fibrin sealant derived from snake venom: from bench to bedside - an overview." J Venom Anim Toxins Incl Trop Dis **23: 21.**


UNICAMP

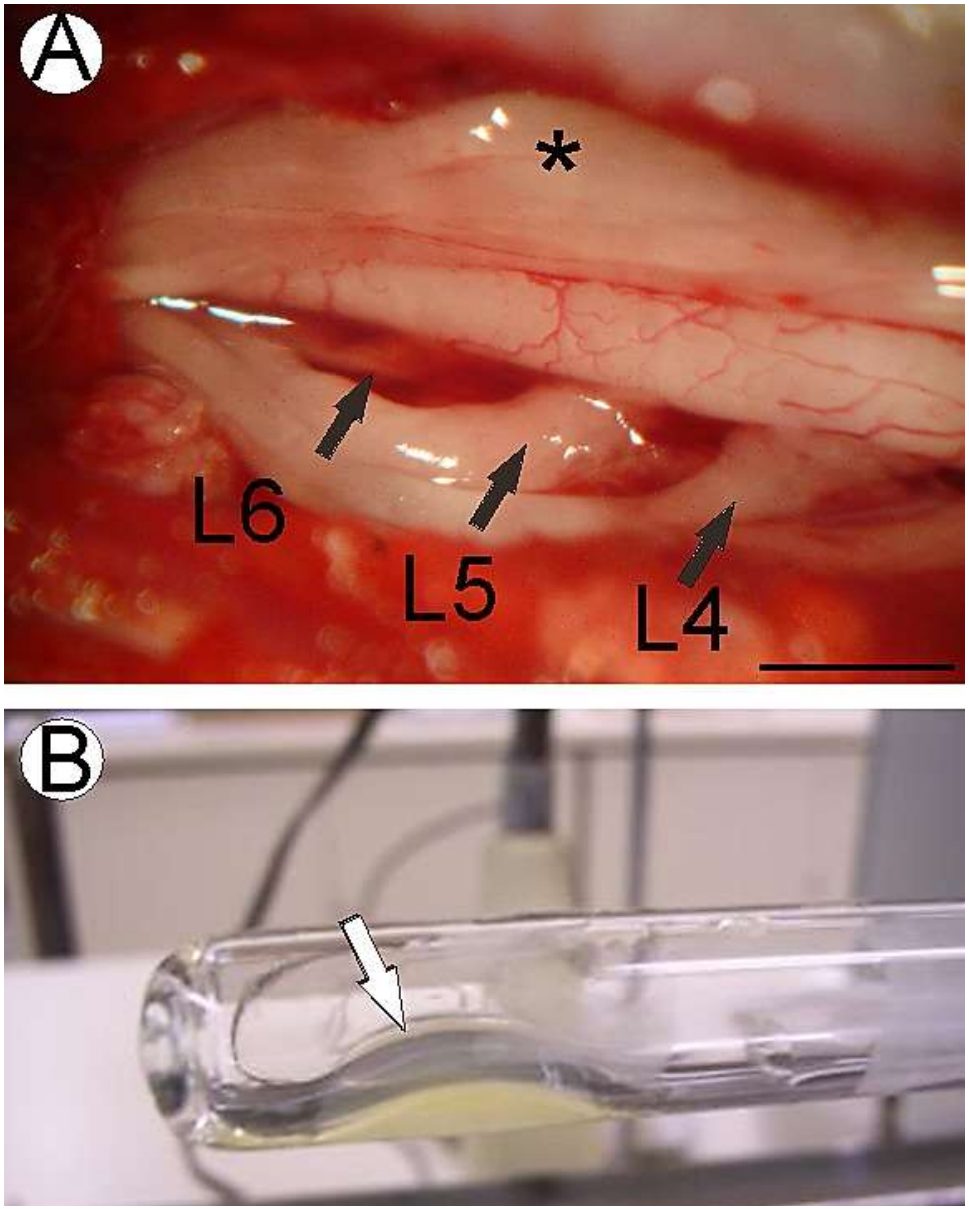
https://scontent.fiev12-1.fna.fbcdn.net/v/t31.0-8/12525531_10154013824934402_2023316150168296705_o.jpg?_nc_cat=100&_nc_ht=scontent.fiev12-1.fna&oh=f8df15dce80f5a351af7a6b77d460c01&oe=5C4379C8

https://www.unicamp.br/unicamp/sites/default/files/banco_imagens/2018/05/18/20180420_2-4_alexandre-leite-rodrigues-de-0liveira-celula-tronco-IB_scarpa_AJS_4173.jpg

Laboratório de Regeneração Nervosa

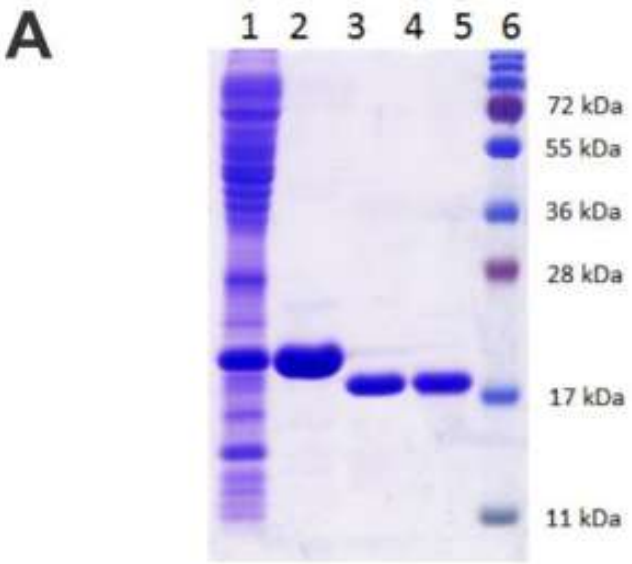


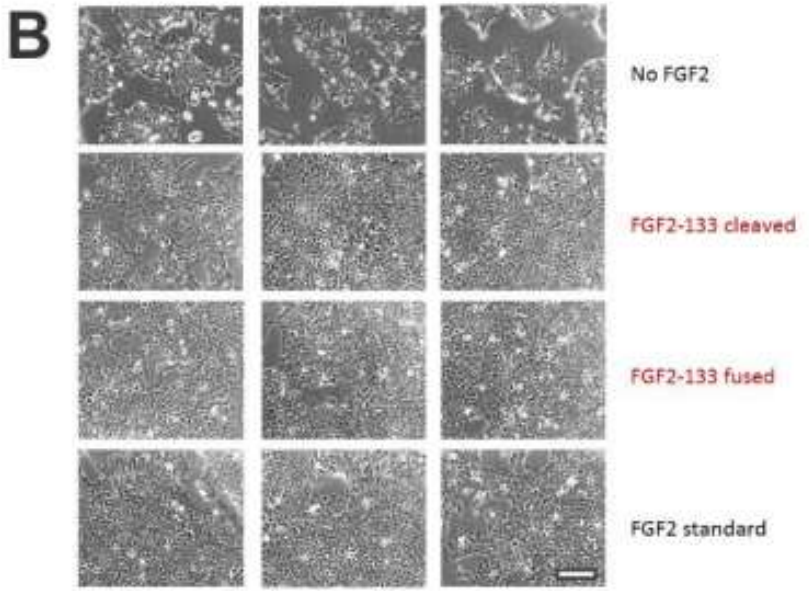
Biscola, N. P., et al. (2017). "Multiple uses of fibrin sealant for nervous system treatment following injury and disease." J Venom Anim Toxins Incl Trop Dis **23: 13.**

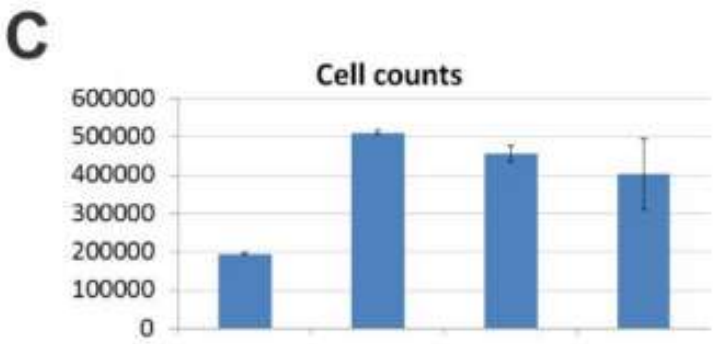


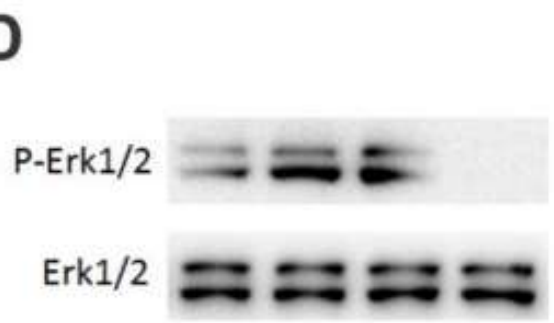
Motor recovery and synaptic preservation after ventral root avulsion and repair with a fibrin sealant derived from snake venom. **Barbizan** R, Castro MV, Rodrigues AC, Barraviera B, Ferreira RS, **Oliveira** AL. PLoS One. 2013 May 7;8(5):e63260.

Supplementary figures and legends









Araujo, M. R., et al. (2017). "Transgenic human embryonic stem cells overexpressing FGF2 stimulate neuroprotection following spinal cord ventral root avulsion." Exp Neurol **294: 45-57.**

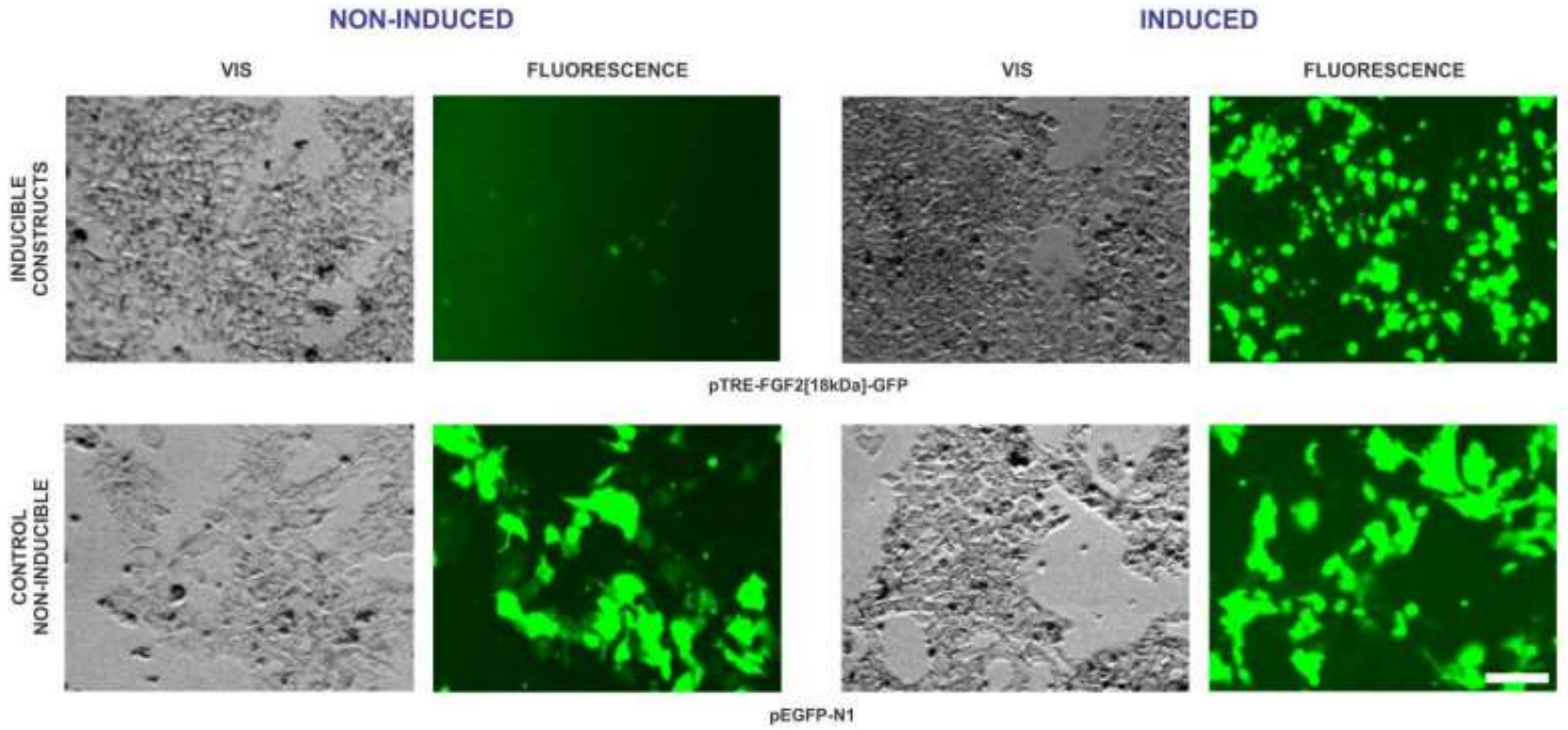


Figure S2. Inducible overexpression of the FGF2-GFP fusion in hESCs. Cells CCTL14 were transiently transfected with the vectors indicated. 1 μM of DOX was added 24 hrs post-transfection and incubation continued for 48 hrs. Scale bar = 200 μm.

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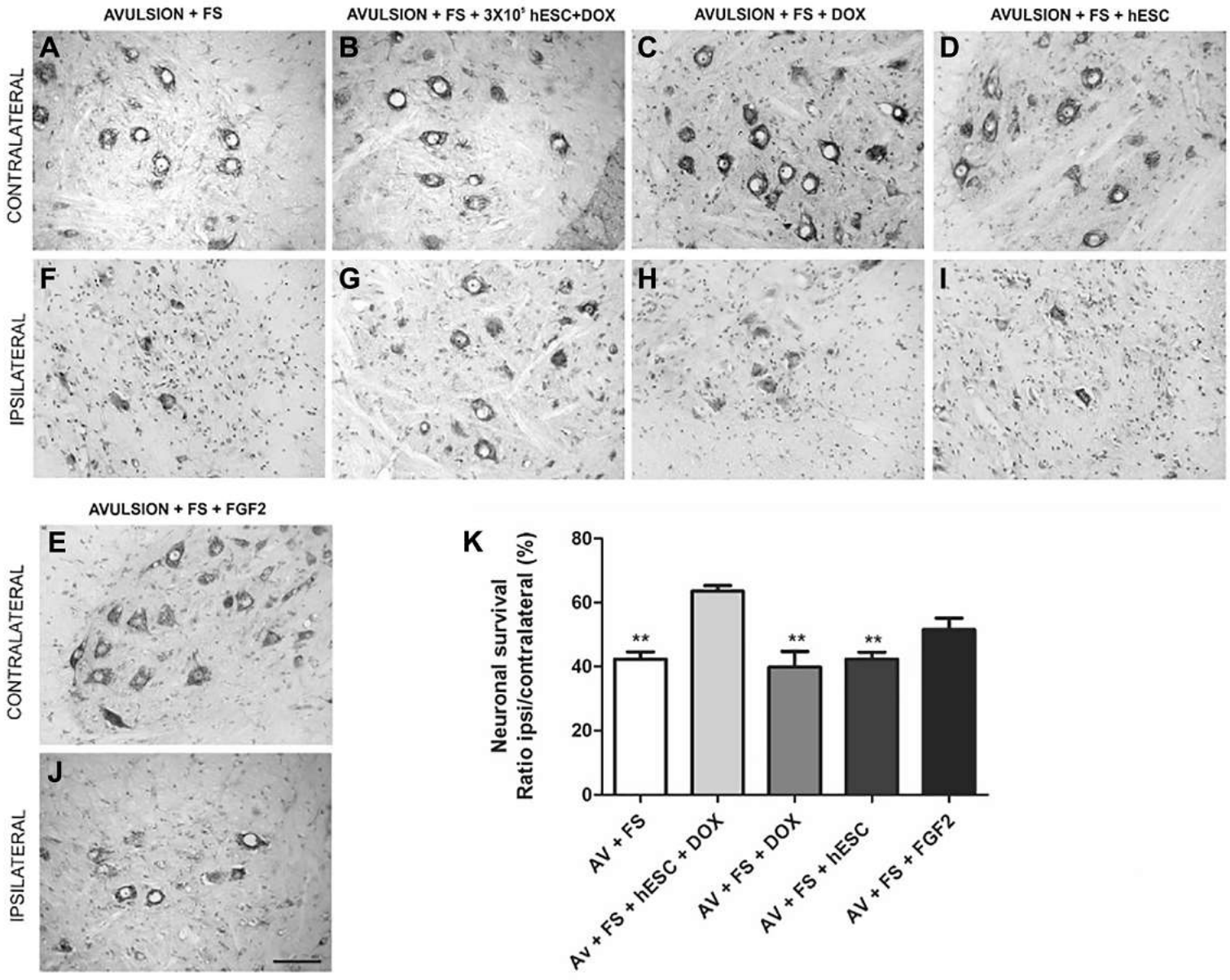

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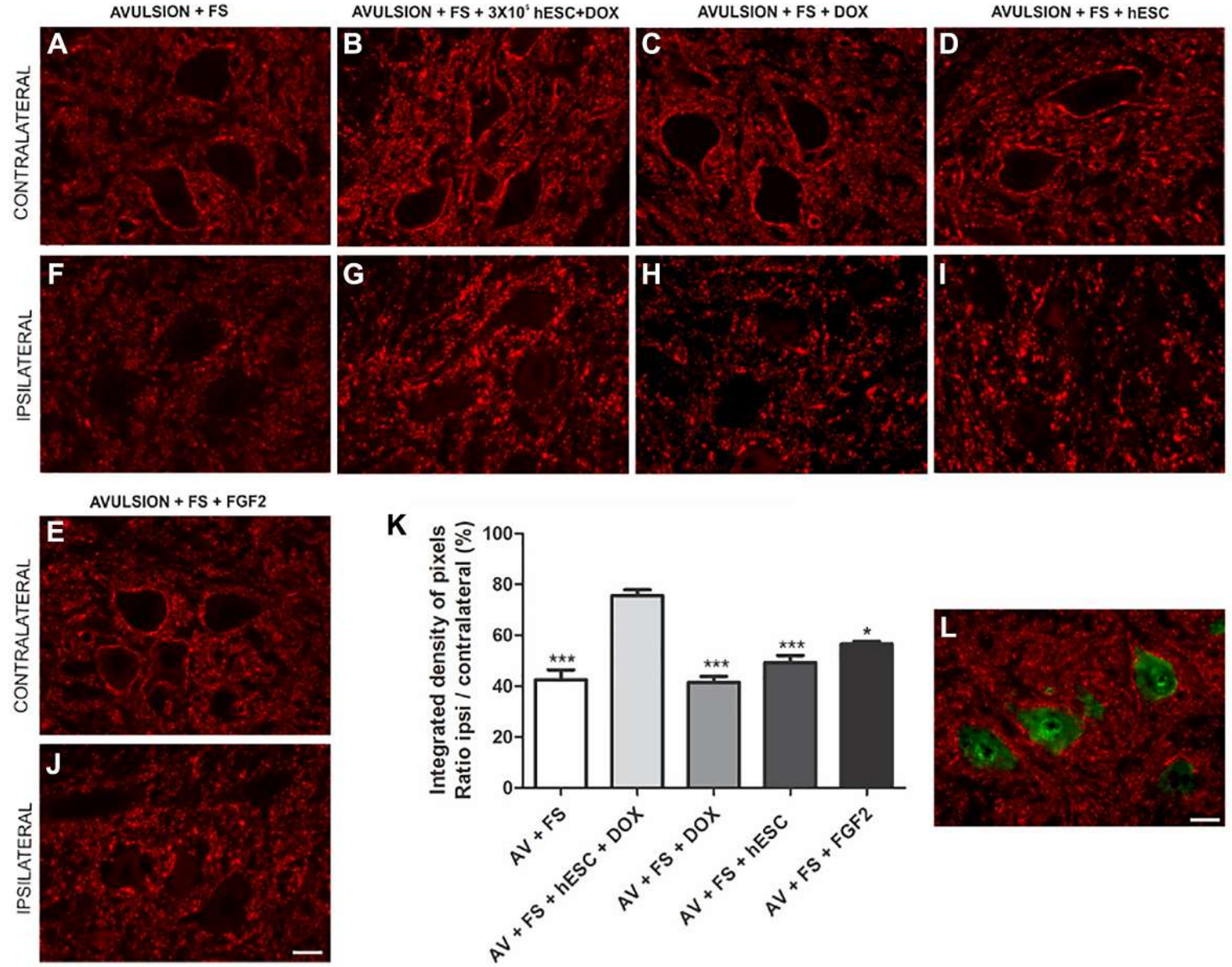


Fig. 4. Immunohistochemical analysis, 2 weeks after avulsion, in the ventral horn of the spinal cord labeled with anti-synaptophysin antibody. (A–E) Contralateral side to lesion. (F–J) Detachment of synaptic terminals on the ipsilateral side. A significant preservation of synaptophysin immunoreactivity was observed in groups treated with hESC + DOX and FGF2. (K) The graphs indicate the ipsi/contralateral ratio of the integrated density of pixels in all groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ relative to AV + FS + hESC + DOX group. Mean ± SE, n = 5 per group. (L) Double immunolabeling of motoneurons present in the lamina IX of Rexed. Synaptophysin staining (red) is combined with NeuN (green), demonstrating that large neuron cell bodies correspond to large motoneurons. Scale bar = 50 μm. AV: avulsion; FS: fibrin sealant; hESC: human embryonic stem cells; DOX: doxycycline. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

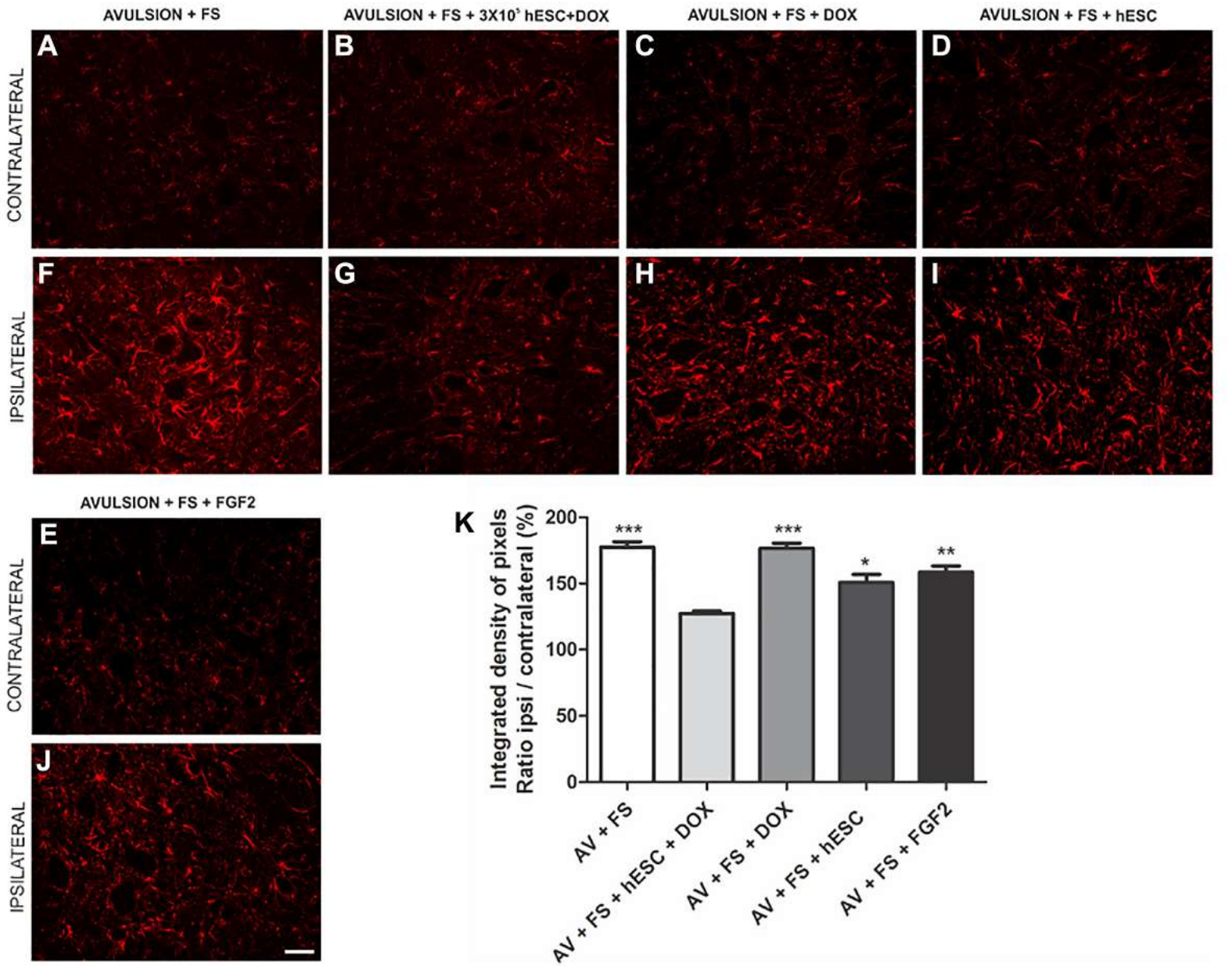


Fig. 5. Immunohistochemical analysis, 2 weeks after avulsion, in the ventral horn of the spinal cord stained with anti-glial fibrillary acid protein (GFAP) antibody. (A–E) Contralateral side. (F–J) Increase in astrogliosis after ventral root avulsion was observed in the ipsilateral side to lesion. A significant reduction of astroglial immunoreactivity was observed in group treated with hESC + DOX. (K) The graph indicates the ratio ipsi/contralateral of the integrated density of pixels in all groups. *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$ relative to AV + FS + hESC + DOX group. Scale bar = 50 μm. Mean ± SE, n = 5 per group. AV: avulsion; FS: fibrin sealant; hESC: human embryonic stem cells; DOX: doxycycline.

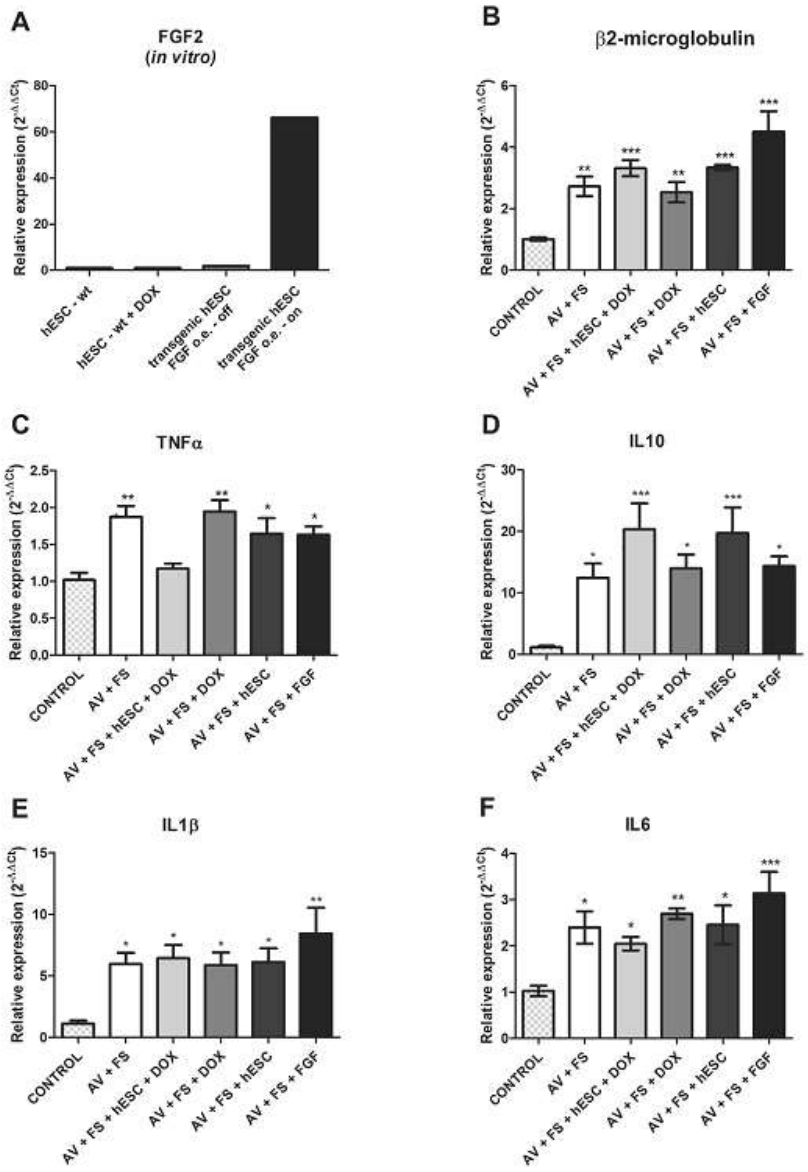
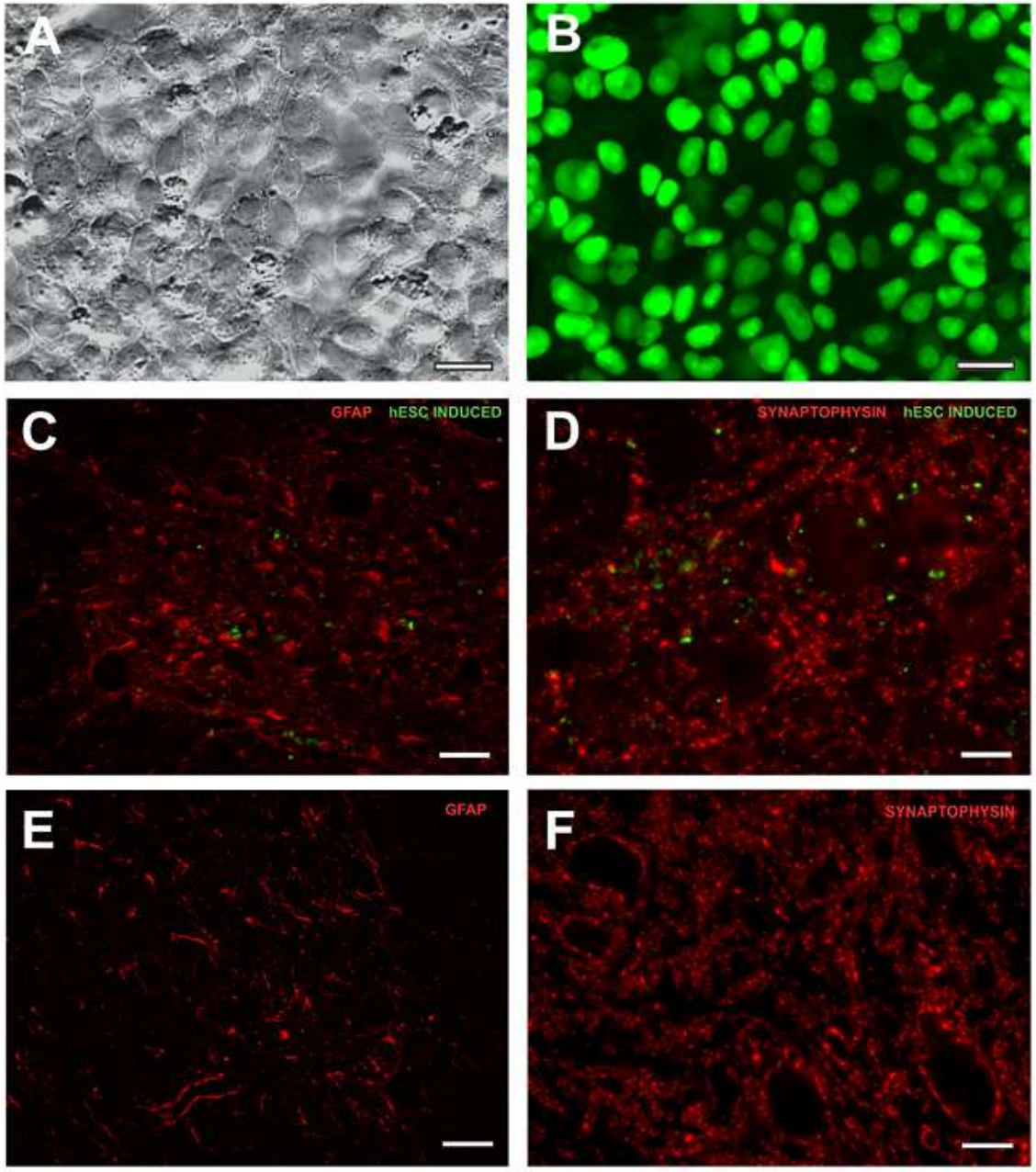


Fig. 7. Relative expression of basic fibroblast growth factor (FGF2) mRNA *in vitro*, and β2-microglobulin (β2m), tumor necrosis factor alpha (TNFα), interleukin 10 (IL10), interleukin 1β (IL1β) and interleukin 6 (IL6) *in vivo*. (A) The transcript levels for FGF2 were over 60 fold higher when hESC induced with doxycycline were used (transgenic hESC - FGF overexpression (o.e.) – on, i.e. doxycycline exposed). In this case, transgenic hESC were compared to wild-type cells exposed or not to doxycycline (hESC wt and hESC + DOX, respectively). Transgenic hESC not treated with doxycycline (FGF o.e. off) presented close to absent expression of FGF2 transcripts. (B) The group treated with FGF2 showed higher expression of β2m as compared to AV + SF and AV + SF + DOX groups. (C) Significant decrease in TNFα expression in the group treated with stem cells overexpressing FGF2. (D) The groups treated with stem cells showed a tendency to higher expression of IL10. (E, F) The expression of these cytokines increased significantly in all treated groups compared to the control group, but there was no significant difference between the injured groups. $^{*}p < 0.05$, $^{**}p < 0.01$; $^{***}p < 0.001$ relative to control group (unlesioned spinal cord). Scale bar = 50 μm. Mean ± SE, n = 5 per group. AV: avulsion; FS: fibrin sealant; hESC: human embryonic stem cells; DOX: doxycycline.



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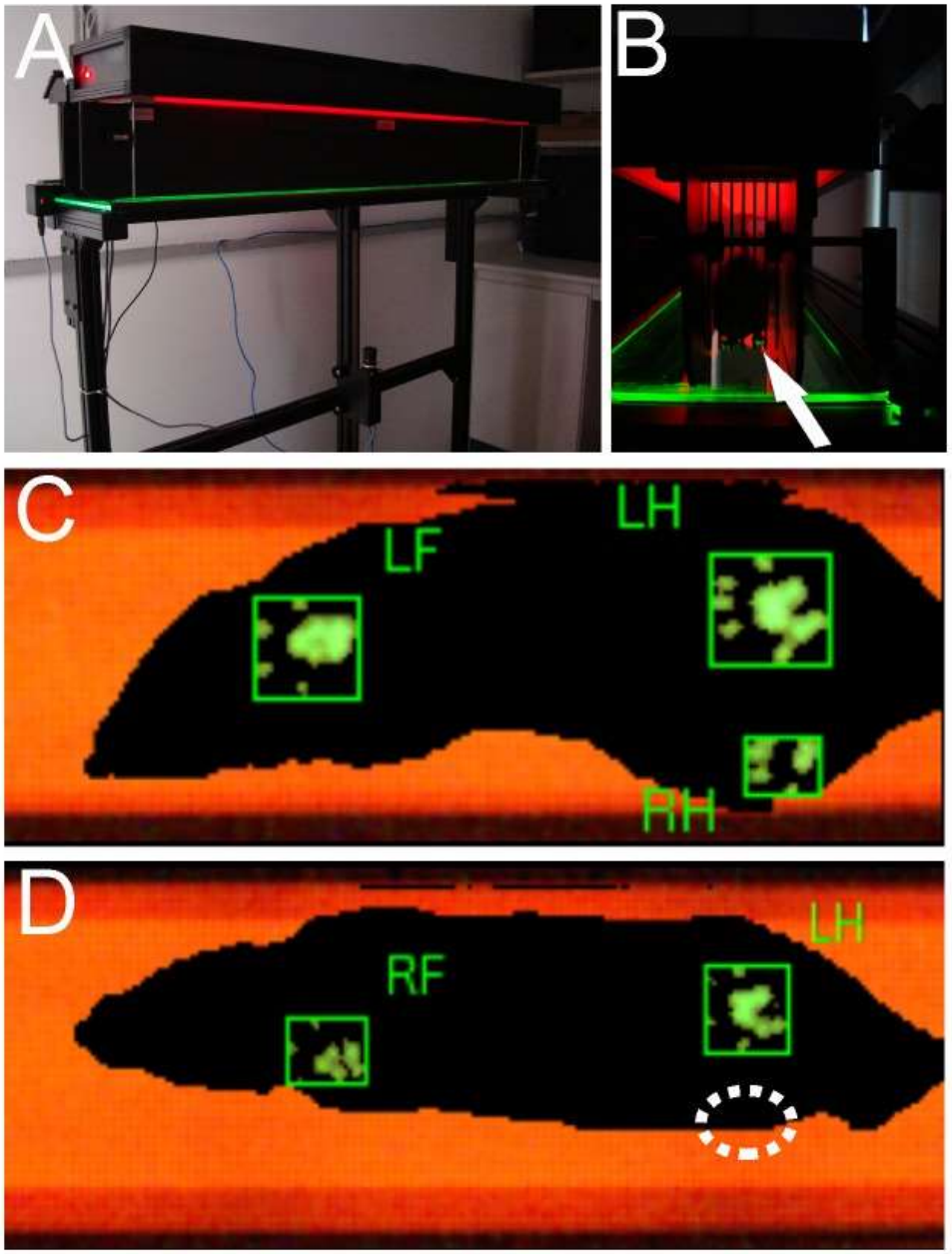

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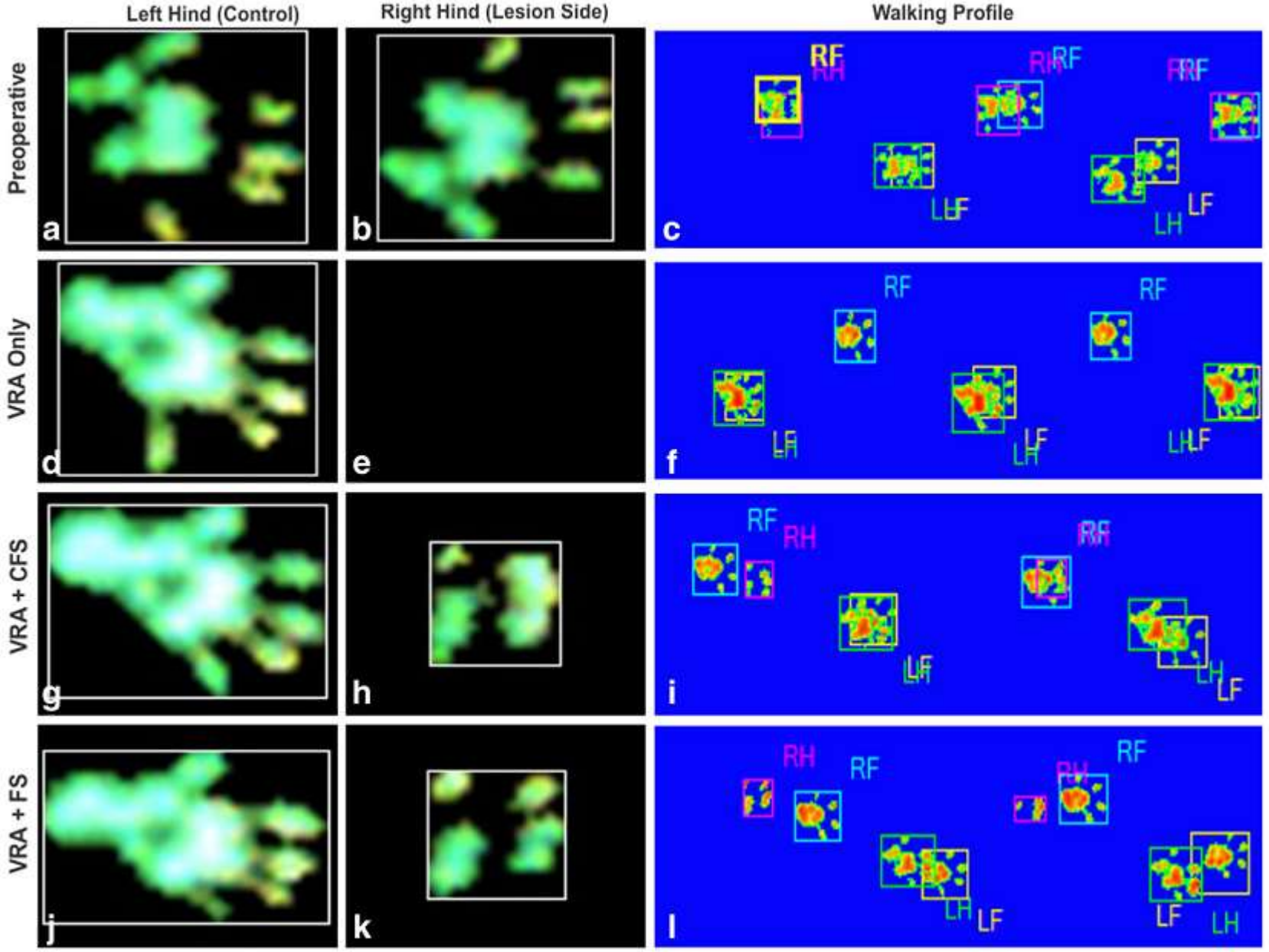

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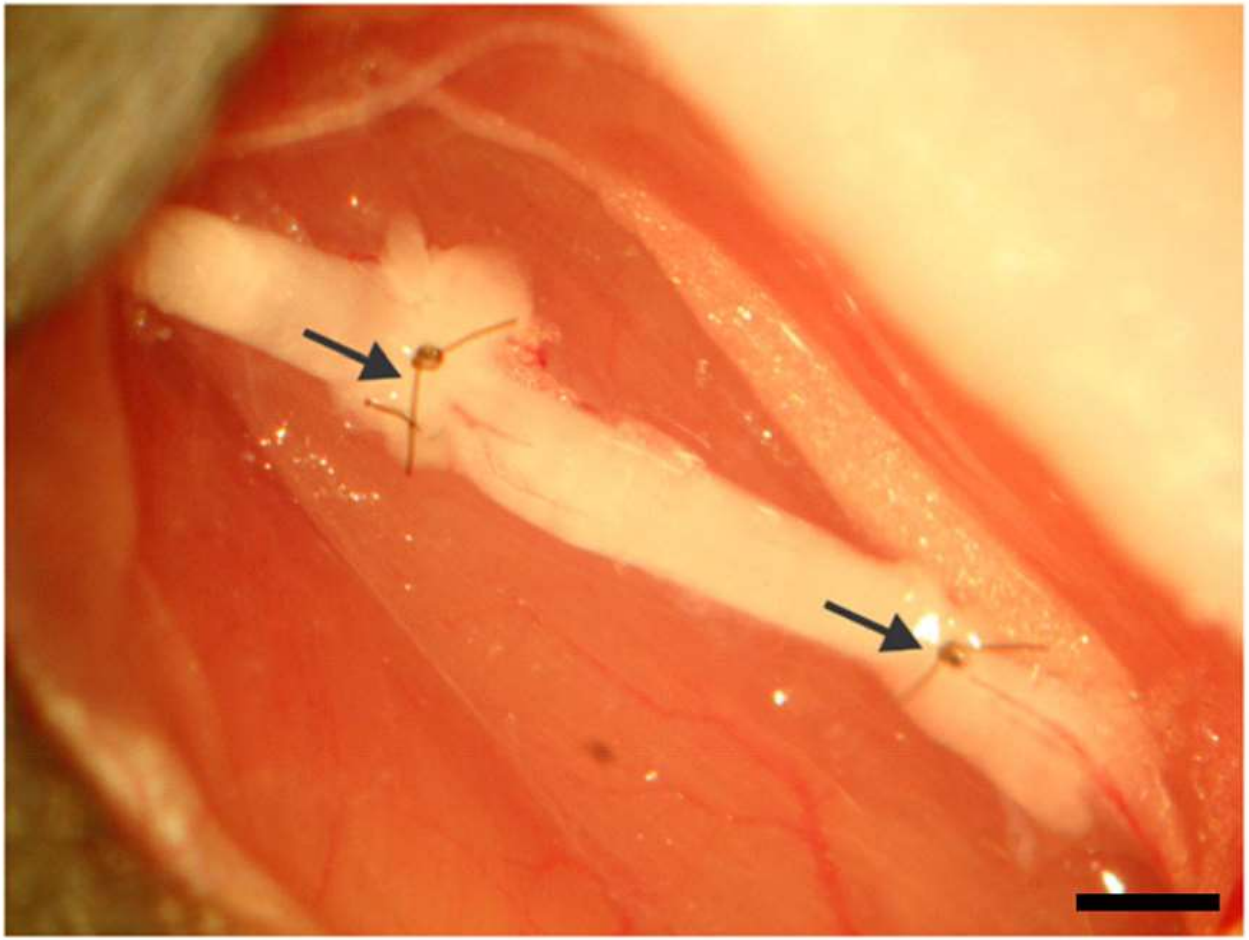

Fig. 1 Autografting procedure in which 5 mm of the sciatic nerve of a mouse is transected, rotated 180 degrees, and then is sutured or stitched together by nylon suture and fibrin sealant (20× magnification). Scale bar: 1 mm

Combination of heterologous fibrin sealant and bioengineered human embryonic stem cells to improve regeneration following autogenous sciatic nerve grafting repair.

Mozafari R, **Kyrylenko S**, Castro MV, Ferreira RS Jr, Barraviera B, Oliveira ALR.

J Venom Anim Toxins Incl Trop Dis. 2018 Apr 12;24:11. doi: 10.1186/s40409-018-0147-x.

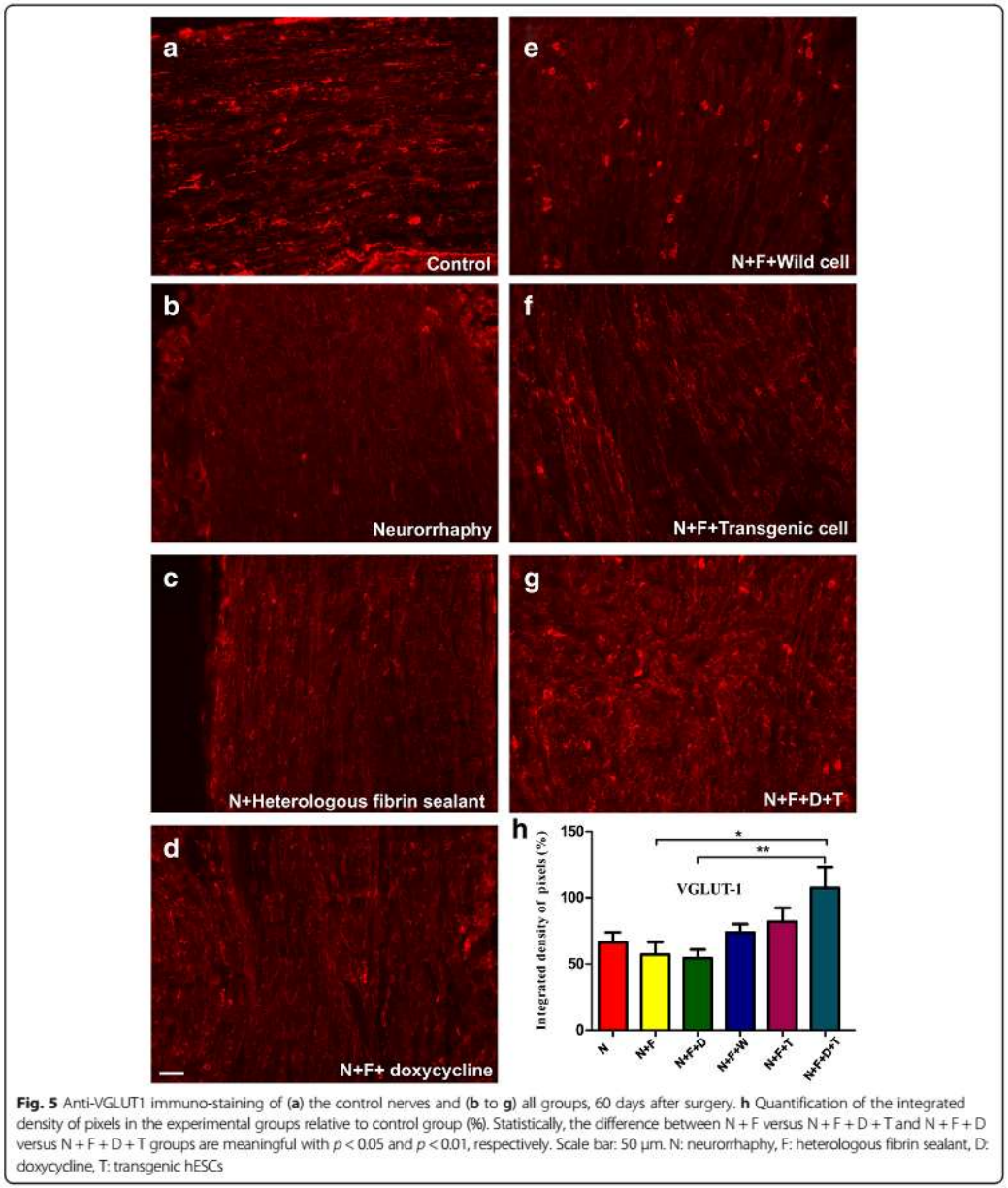

Fig. 5 Anti-VGLUT1 immuno-staining of (**a**) the control nerves and (**b** to **g**) all groups, 60 days after surgery. **h** Quantification of the integrated density of pixels in the experimental groups relative to control group (%). Statistically, the difference between N + F versus N + F + D + T and N + F + D versus N + F + D + T groups are meaningful with $p < 0.05$ and $p < 0.01$, respectively. Scale bar: 50 μm. N: neurorrhaphy, F: heterologous fibrin sealant, D: doxycycline, T: transgenic hESCs

[Combination of heterologous fibrin sealant and bioengineered human embryonic stem cells to improve regeneration following autogenous sciatic nerve grafting repair.](#)

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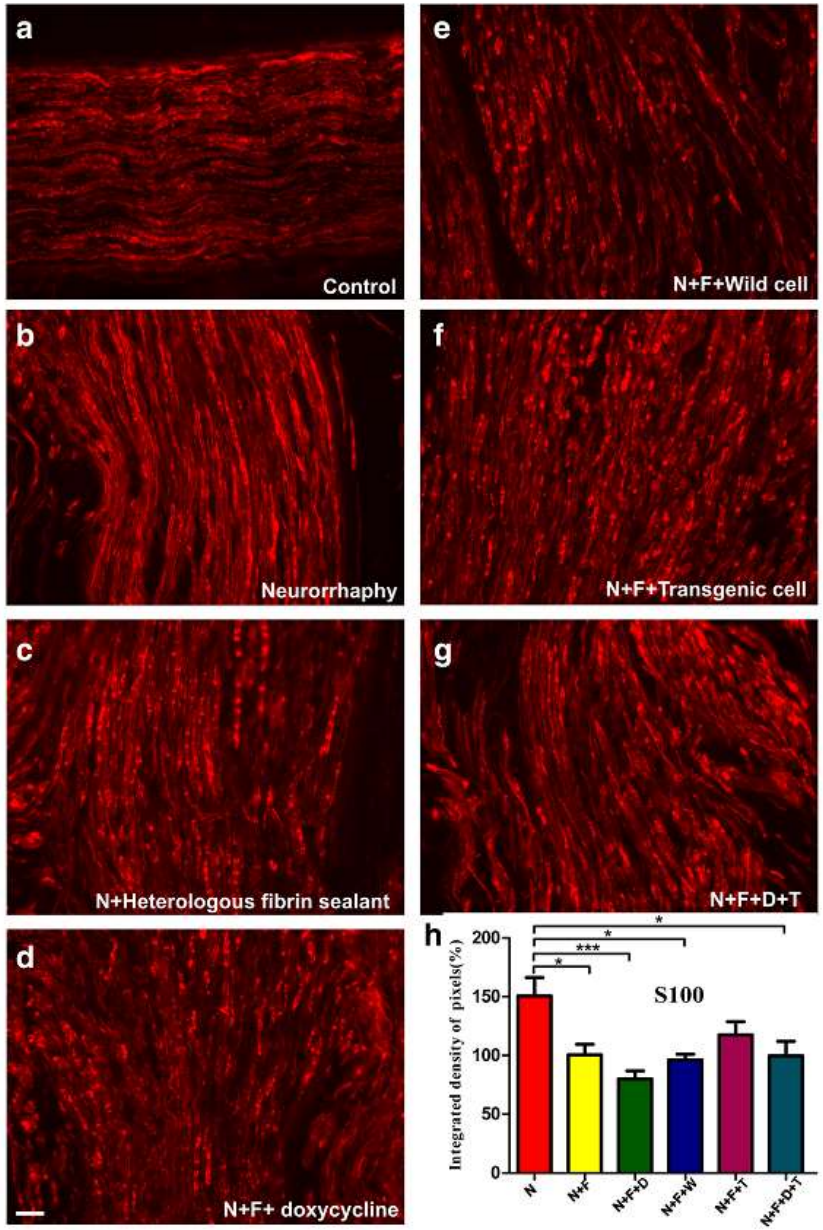

Fig. 6 Anti-S100 immuno-staining of (**a**) the control nerves and (**b** to **g**) all groups, 60 days after surgery. **h** Quantification of the integrated density of pixels in the experimental groups relative to control group (%). Statistically, the difference between the following groups are meaningful: N versus N + F ($p < 0.05$), N versus N + F + D ($p < 0.001$), N versus N + F + W ($p < 0.05$), and N versus N + F + D + T ($p < 0.05$). The N versus N + F + T shows no significant difference. Scale bar: 50 μm. N: neurorrhaphy, F: heterologous fibrin sealant, D: doxycycline, T: transgenic hESCs

Mozafari R et al 2018

Future pharmacology:

- chemically defined
- animal - free

https://ask.extension.org/uploads/question/images/attachments/000/033/553/image_original.jpg?1472595398

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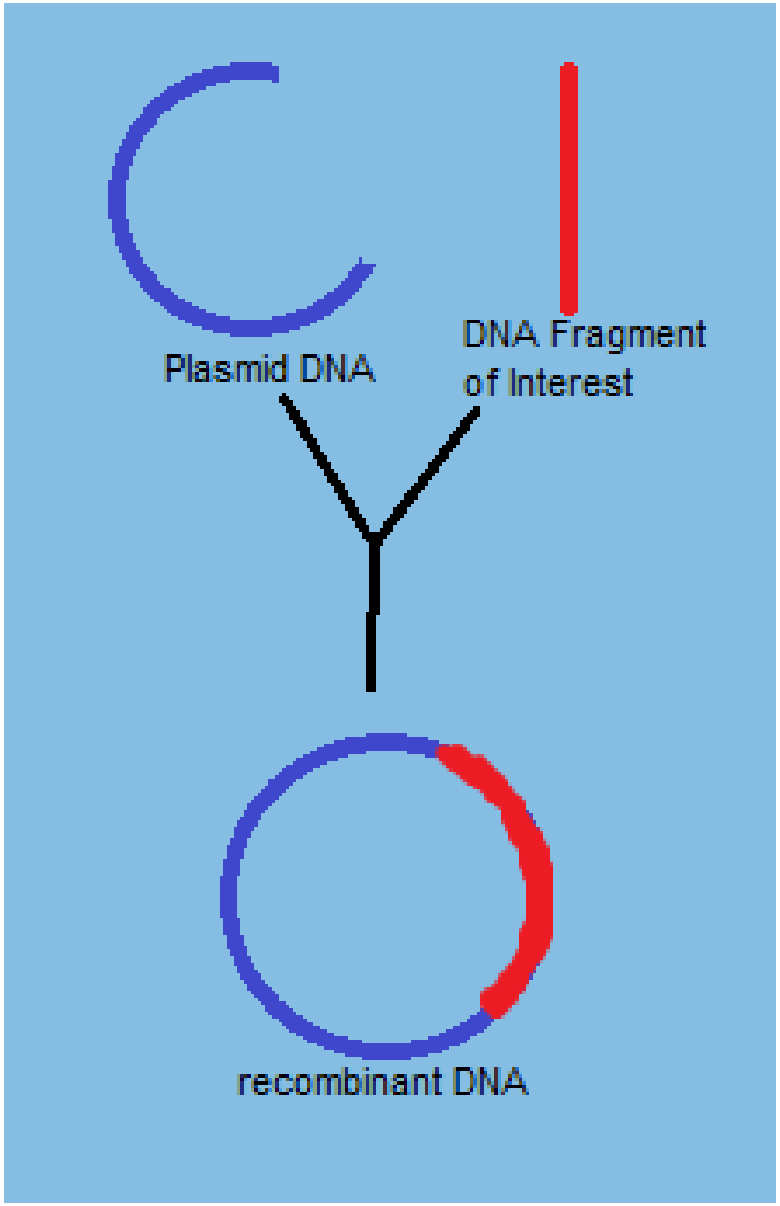

http://genomeed.weebly.com/recombinant-dna.html

Advantages of gyroxin over thrombin:

- not inhibited by antithrombin, heparin, warfarin, hirudin and others;
- activity is constitutive;
- independent on Calcium;
- does not activate protease-activated receptors (PARs).

Coughlin SR et al 2005
Yonamine CM et al 2014
Bjork and Lindahl 1982

Recombinant Gyroxin, rG

- activity
- specificity
- stability
- technological feasibility
- capacity for engineering

- **„ consumptive snakepathy'' in nature ☺**


1032
БІЛА
ЦЕРКВА


biopharma

компанія виробництво продукція фармаконагляд тендери контакти

biopharma — імунобіологічна фармацевтична компанія

Костянтин Єфименко
президент компанії

Ми розробляємо і виробляємо препарати з плазми донорської крові людини, рекомбінантні препарати, пробіотики на основі спороутворюючих бактерій і традиційні лікарські засоби.

https://www.biopharma.com.ua/kompaniya-2/

… According to current contracts, Kedrion Biopharma provides for the manufacturing of the following plasma-derived medicinal products (PDMPs) :

- human albumin solution (albumin),
- polyvalent immunoglobulin for intravenous administration (IVIG),
- factor VIII concentrates (FVIII),
- factor IX concentrates (FIX),
- prothrombin complex concentrates (PCCs)
- and antithrombin (AT).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853983/

Plasma-derived medicinal products and their clinical indications:

- **Human albumin**
- **Immunoglobulins (IG)**
- **Antithrombin**
- **Coagulation Factor VIII concentrates (FVIII)**
- **Coagulation factor IX concentrates (FIX)**
- **Coagulation factor VII concentrates (FVII)**
- **Coagulation factor XIII (FXIII)**
- **Prothrombin Complex Concentrates (CCP)**
- **Fibrinogen**

http://www.italiaplasma.it/en/medicinali-plasmaderivati/

Фармацевтическая компания "Биофарма" (Киевская обл.) в рамках реализации проекта по реконструкции Сумской областной станции переливания крови (СПК) намерена восстановить институт кадровых доноров для получения качественного сырья для производства препаратов крови.

"Сегодня мы формируем институт кадровых доноров... Главным образом, потому что нам нужно получить качественные компоненты крови для больниц и плазму для завода фракционатора. Не секрет, что в Украине очень высокий процент вирусных заболеваний, таких как ВИЧ-инфекция, гепатиты, включая С, туберкулез", - сказал в интервью агентству "Интерфакс-Украина" председатель совета директоров компании "Биофарма" Константин Ефименко.

Он также сообщил, что "Биофарма" в 2015 году начала строительство фракционатора плазмы крови проектной мощностью 400 тонн. В апреле 2016 года

https://interfax.com.ua/news/pharmacy/324721.html

Recombinant systems:

- **bacteria**
- yeast
- cell culture
- protozoa
- **green plants**

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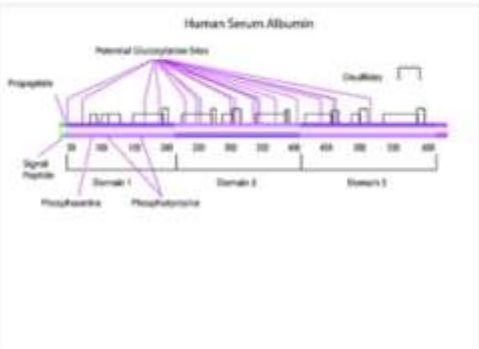
A9731 Sigma

Albumin human

recombinant, expressed in rice, lyophilized powder, cell culture tested, low endotoxin, ≥96% (Excised bands analyzed by MS to yield total albumin content, PAGE)

Synonym: **Cellastim™, rHSA**

CAS Number 70024-90-7 | MDL number MFCD00081418 | eCl@ss 42010201



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JB THE JOURNAL OF BIOCHEMISTRY

High-level expression and preparation of recombinant human fibrinogen as biopharmaceuticals

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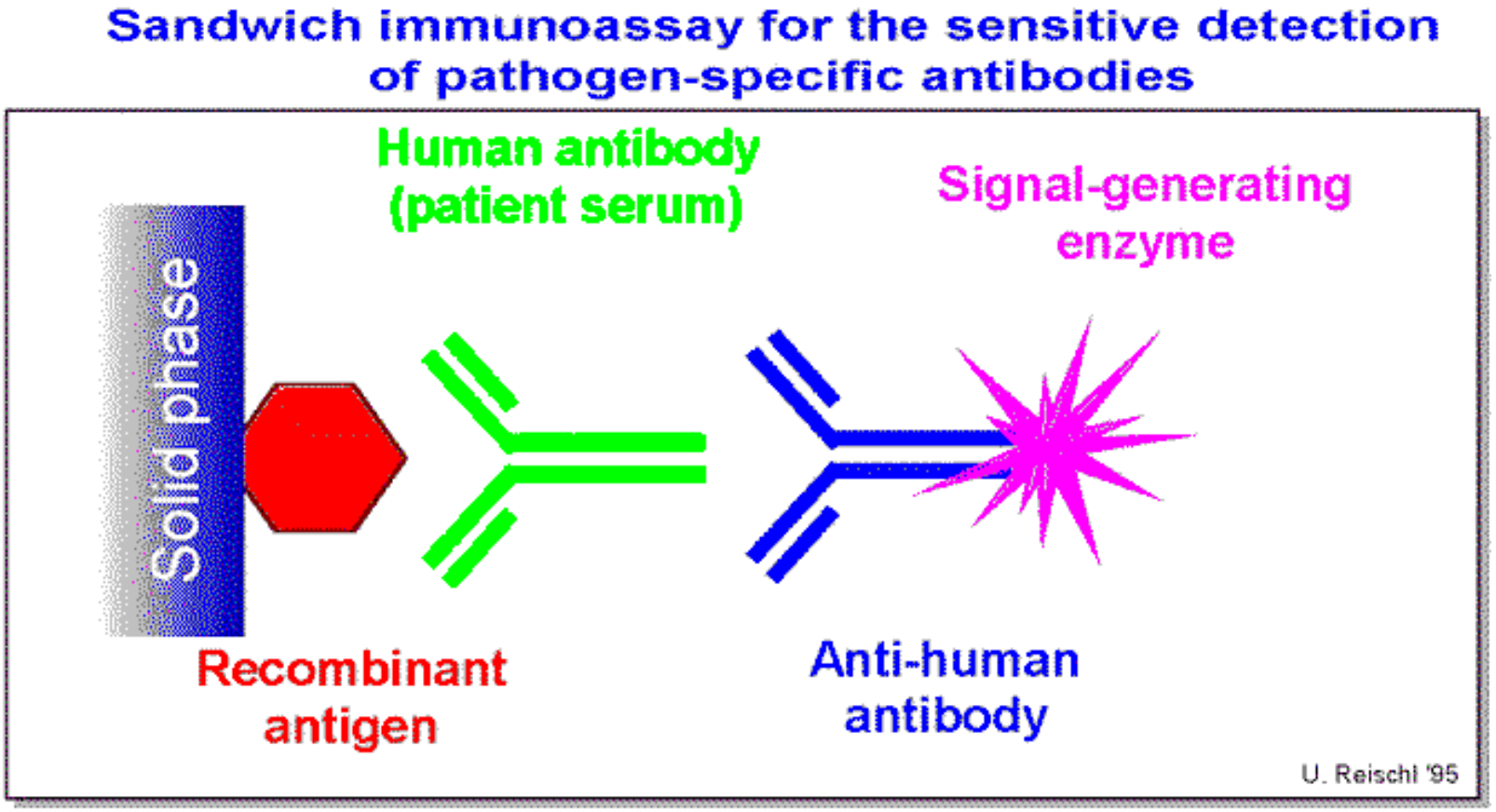
Fibrinogen is a large and complex glycoprotein containing two sets of each of three different chains (α, β and γ). There have been no reports of high-level expression of fibrinogen at commercial levels using mammalian cultured cells such as CHO cells because of the difficulty in highly expressing a protein with such a complex structure. We achieved high-level (1.3 g/l or higher) expression of recombinant human fibrinogen using CHO DG44 cells by optimizing the expression system and culture conditions. We also succeeded in establishing a high-recovery preparation method for recombinant fibrinogen that rarely yields degraded products. To characterize the properties of the recombinant

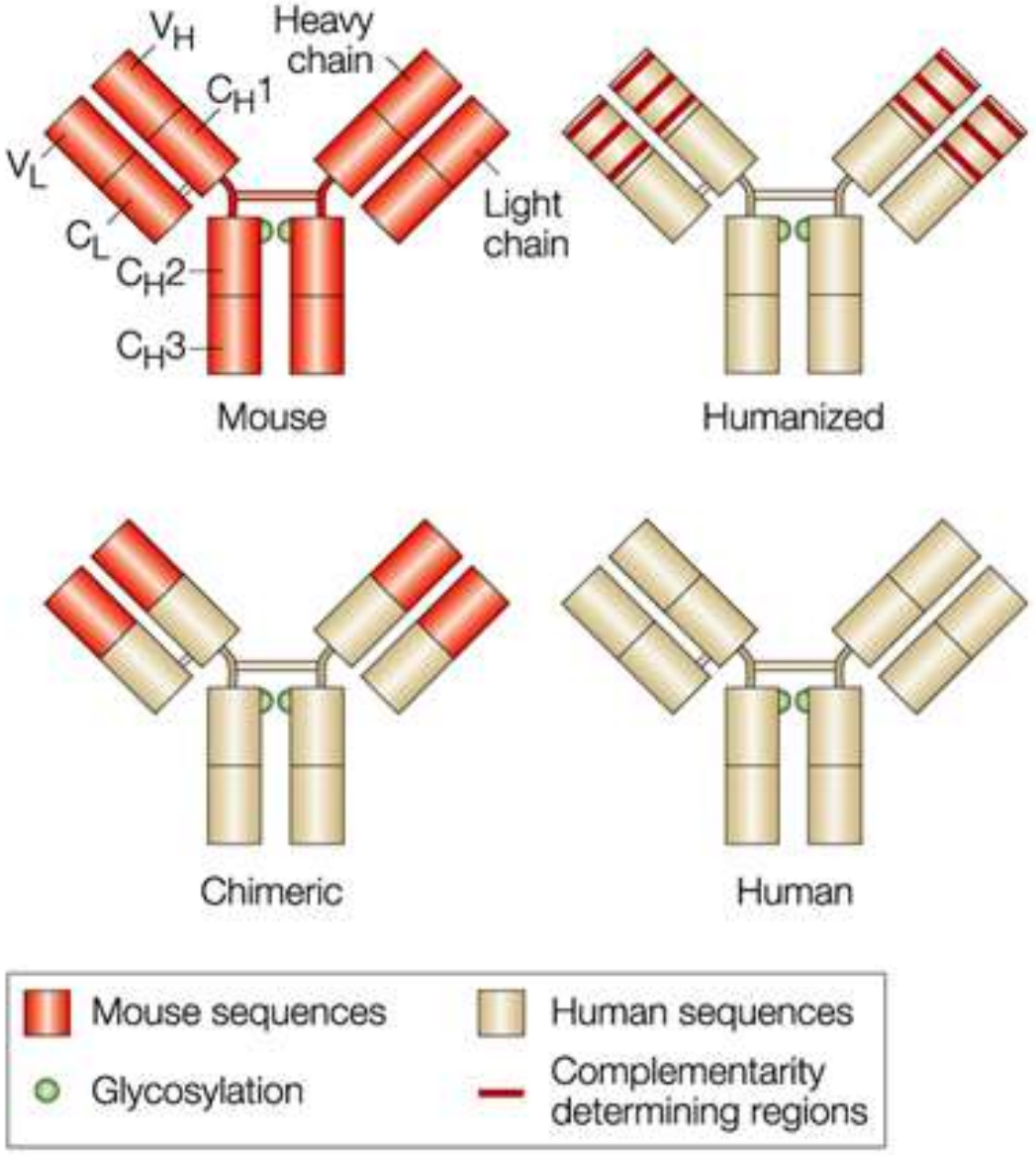
Many blood plasma products such as albumin, globulin and coagulation factors, including fibrinogen and thrombin, are used therapeutically. Although recombinant albumin, several monoclonal antibodies and some coagulation factors are available as biopharmaceuticals (*1–3*), production of fibrinogen for this purpose has not been successful.

Fibrinogen is a clotting factor that acts in the final stage of the blood coagulation cascade and is converted into a fibrin monomer by activated thrombin. The fibrin monomers then polymerize in the presence of coagulation factor XIII (FXIII) and calcium to form fibrin polymers, which are precipitated as fibrin fibrils in tissue (*4*). These insoluble fibrin clots and aggregated platelets constitute a thrombus, which leads to haemostasis. Fibrin sealants based on this clotting action have been used for more than 20 years as surgical haemostatic and sealing agents (*5*, *6*).

Fibrinogen, a non-spherical protein with a large molecular size of 340 kDa, is susceptible to physical shearing and degradation by proteases in the manufacturing process because of its structural characteristics. Degraded fibrinogen cannot form a strong polymer structure even when transformed to fibrin, resulting in the failure of haemostasis (*7–9*). Although fibrinogen is not degraded, if it is not controlled with appro-



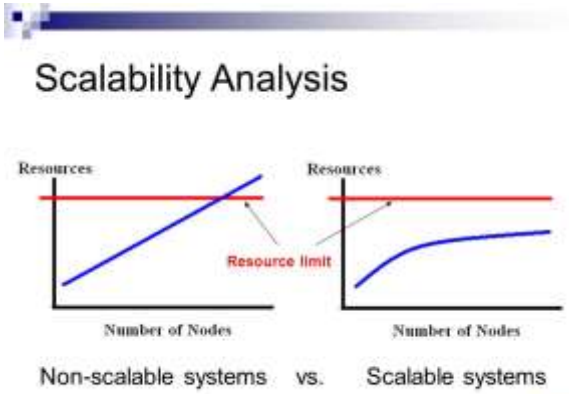
https://www.bioscience.org/1996/v1/e/reischl1/images/fig2.gif



http://neobiolab.com/research/wp-content/uploads/2015/10/Antibodies.gif

Human donor blood

is a non-scalable resource!!!



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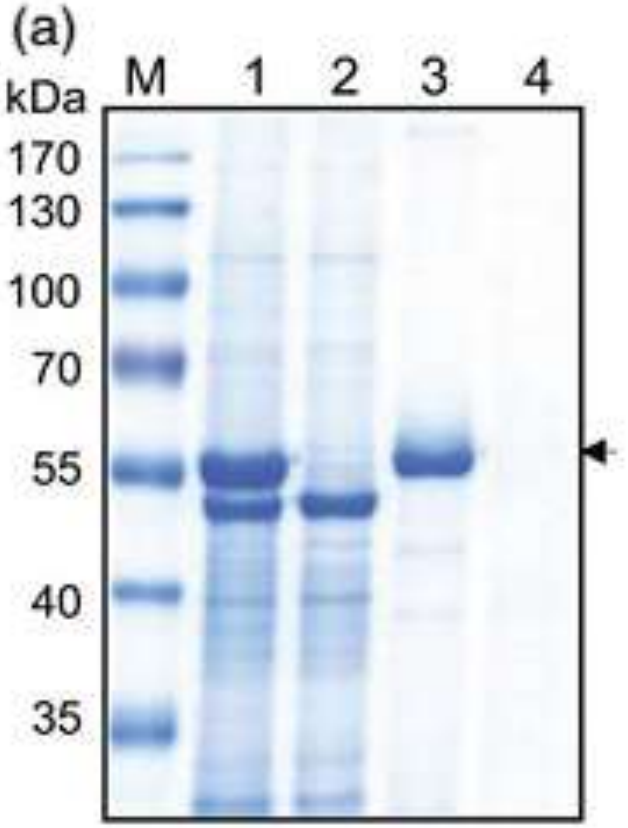
Erasmus+
Jean Monnet Modules

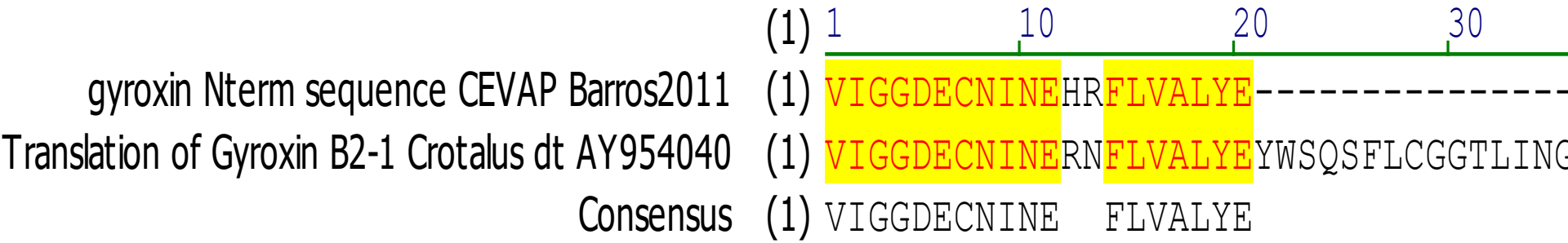


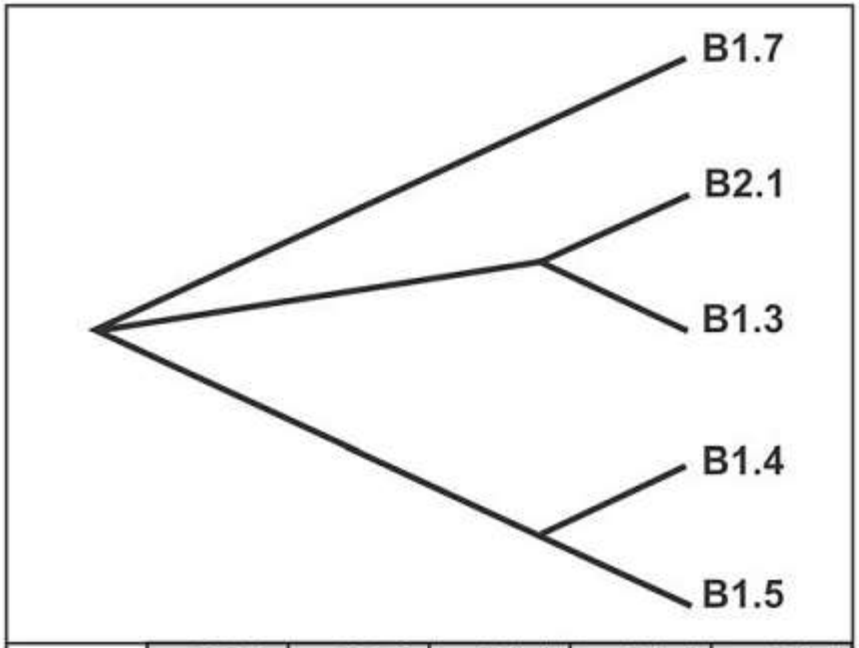
b-glucosidase Bgl4

http://img.archiexpo.com/images_ae/photo-g/65375-1857921.jpg

Hahn S et al, 2015

Aligment of Guroxin from CEVAP (Barros 2011) and Gyroxin B2-1 from Butantan SP



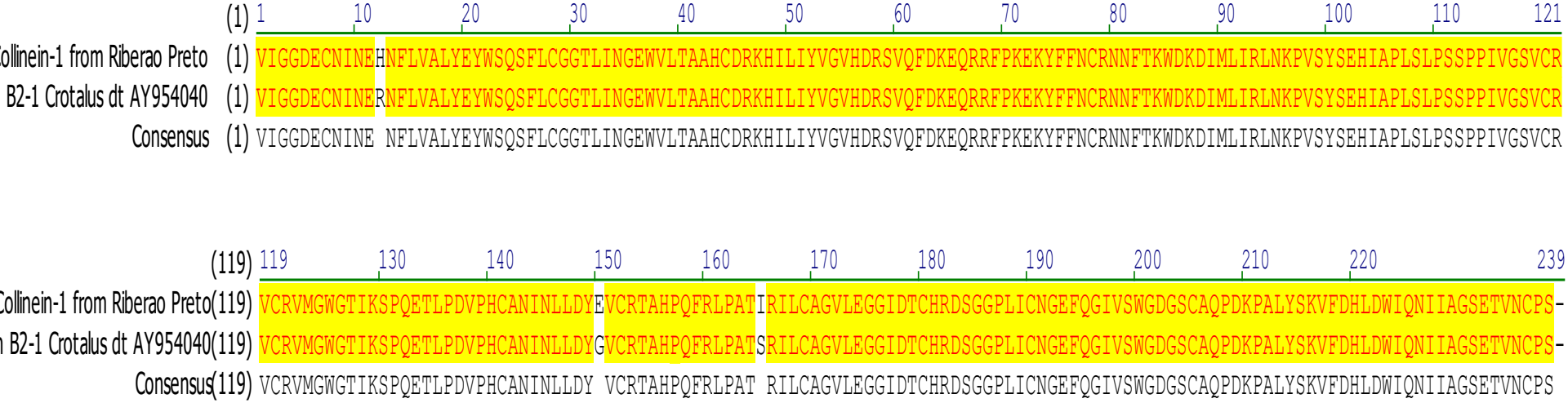


	B2.1	B1.3	B1.4	B1.5	B1.7
B2.1	100.00	99.55	94.40	94.86	84.04
B1.3	99.55	100.00	96.14	96.67	88.45
B1.4	94.40	96.14	100.00	99.76	88.57
B1.5	94.86	96.67	99.76	100.00	89.57
B1.7	84.04	88.45	88.57	89.57	100.00

Fig. 2.Similarity of cDNAs sequences of clones B2.1, B1.3, B1.4, B1.5 and B1.7. The table shows the similarity of percentage among the clones amplified from Crotalus durissus terrificusvenom gland. Dendrogram was made from the alignment of cDNA sequences using PHYLYP 3.6 program.

Yonamine CM[1], Prieto-da-Silva AR, Magalhães GS, Rádis-Baptista G, Morganti L, Ambiel FC, Chura-Chambi RM, Yamane T, Camillo MA. **Cloning of serine protease cDNAs from Crotalus durissus terrificus venom gland and expression of a functional Gyroxin homologue in COS-7 cells.** Toxicon. 2009 Aug;54(2):110-20.

Aligment of Collinein-1 from Riberão Preto and Gyroxin B2-1 from Butantan SP



>gyroxin consensus CEVAP Dec2016

viggdecnine**hn**flvalyeywsqsflcggtl
ingewvltaahcdrkhiliyvgvhdrsvqfdk
eqrrfpkekyffncrnnftkwdkdimlirlnk
pvsysehiaplslpssppivgsvcrvmgwgti
kspqetlpdvphcaninlldy**e**vcrtahpqfr
lpat**S**rilcagvleggidtchrdsggplicng
efqgivswgdgscaqpdkpalyskvfdhldwi
qniiagsetvncps