

6 de Março - Equinos

Técnicas de reabilitação equina

11h00 - Novas perspectivas terapêuticas para a Laminite

Dr. Jean-Philippe Lejeune, REVATIS

11h45 - Medicina regenerativa: a solução mais eficaz para a
recuperação de lesões? - Dr. Jean-Philippe Lejeune, REVATIS



www.revatis.com

Equine regenerative medicine

2

DÉFINITION AND OBJECTIVES

SEVERAL APPROACHES:

MATRIX THERAPY
GROWTH FACTORS
STEM CELLS

Definition

Regenerative medicine deals with the "process of replacing, engineering or regenerating tissues or organs to restore or establish normal function". It aims at stimulating the body's own repair mechanisms.

Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely implant them when the body cannot heal itself.

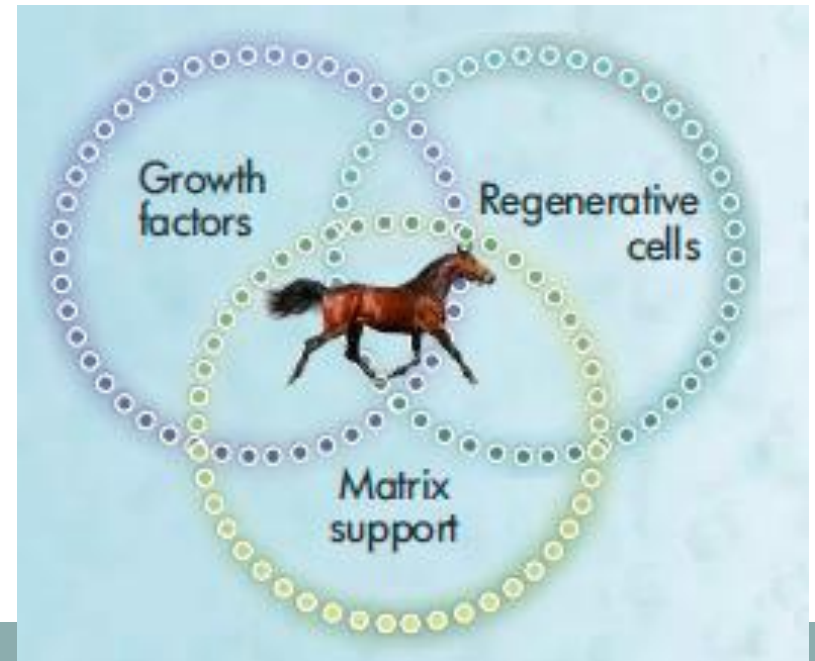
Regenerative medicine refers to a group of biomedical approaches to clinical therapies that may involve the use of stem cells, growth factors and/or matrix support.

Objectives

Cell therapy aims at healing an organ or an organism by using cells obtained most of the time from stem cells in order to replace defective cells.

e.g.: tendonitis – functional repair instead of fibrosis

osteoarthritis – healing instead of hiding





Matrix therapy

Synolis^R (HA + sorbitol)



Context



- Hyaluronic acid: well-known since a few years
- Intra-articular, intra-venous (???) use
- Usually used with corticoids
- « it eases the conscience »

Existing medicines

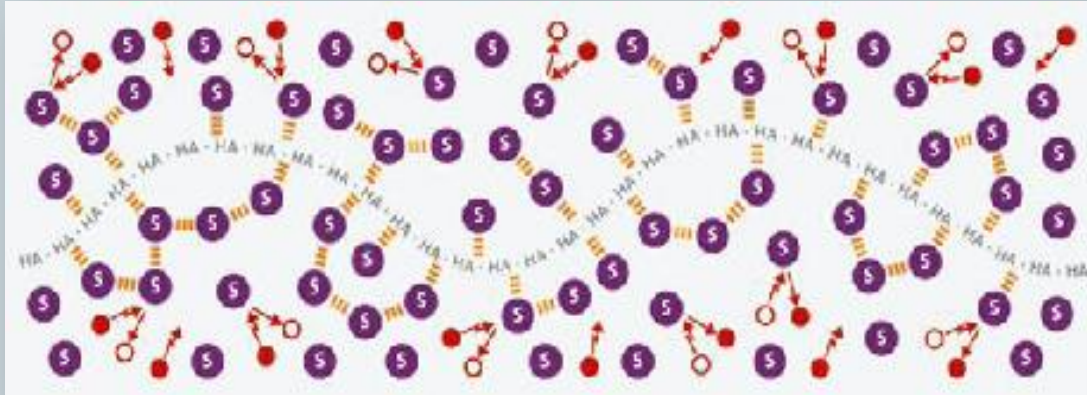
- **HY-50® VET (Dechra)** 17 mg/mL (51mg in a 3 mL syringe) – 120-600 x10³ Da



- **HYONATE (Bayer)** 10 mg/mL (20 mg in a 2 mL syringe)



An alternative: Synolis-VA^R



Matrix support



Mechanism of action

- Synolis V-A contains the latest generation of viscosupplementation for the treatment of osteoarthritis. It is a combination of a 2% solution of hyaluronic acid and a 4% solution of sorbitol.
- Sorbitol as an additive provides
 - Improved viscoelastic properties
 - Improved preservation of hyaluronic acid
 - An anti-oxidant effect

Technical specifications



Hyaluronic acid	20 mg/ml, pharmaceutical grade product obtained from bio-fermentation, molecular weight > 2.2 MDa
Sorbitol	40 mg/ml, pharmaceutical grade
Volume of the syringe	2 ml
Mode of sterilisation	Vapour
Presentation	Sterile glass syringe
Packing	Individual sterile package
Clinical use	Intrasynovial injection
Storage	Ambient temperature

Technical specifications



- Synolis V-A is an approved « medical device class 3 » . It has been developed to improve the comfort of human patients suffering from osteoarticular pathologies.
- Synolis V-A is used in horses under the responsibility of a licensed veterinary surgeon, as there is no equivalent veterinary approved product.

Synolis-VA^R - Clinical studies

- In humans

Int J Immunopathol Pharmacol. 2014 27(2):245-52.

Duration of symptom relief after intra-articular injection of hyaluronic acid combined with sorbitol (anti-ox-vs) in symptomatic hip osteoarthritis.

Migliore et al.

Hip osteoarthritis, a single injection: improvement during

12 months and more in 75 % des patients

9 months in 8.3 % des patients

6 months in 16.7 % des patients



Synolis-VA^R - Clinical studies

• In humans



Drug Res (Stuttg). 2013, 63(9):445-9.

Safety and efficacy findings from a non-interventional study of a new hyaluronic acid/sorbitol formulation (GO-ON® matrix) for intra-articular injection to relieve pain and disability in osteoarthritis patients.

Heisel et al. 1147 patients



Knie osteoarthritis 60 % - a single injection et 40 % 3 injections

No significant difference between the results at 3 and 6 months

	T0	T 6 months
Severe pain	56,20%	5,90%
No pain or light pain	28,9	66,40%
Severe to very severe functional disability	29,1	3,90%

Synolis-VA^R - Clinical studies



- In horses – preliminary study

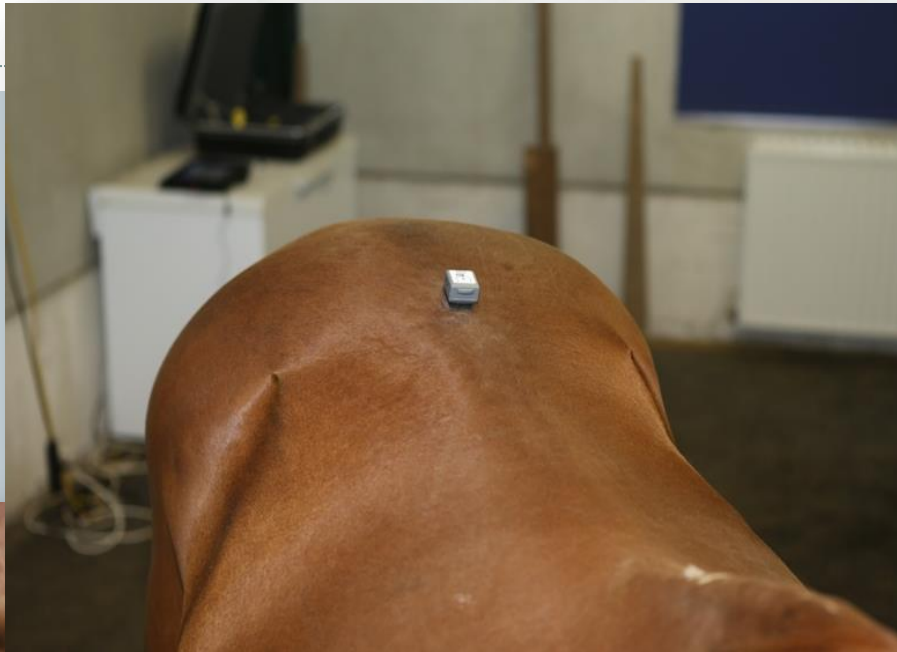
Usually used in vet clinics in Belgium

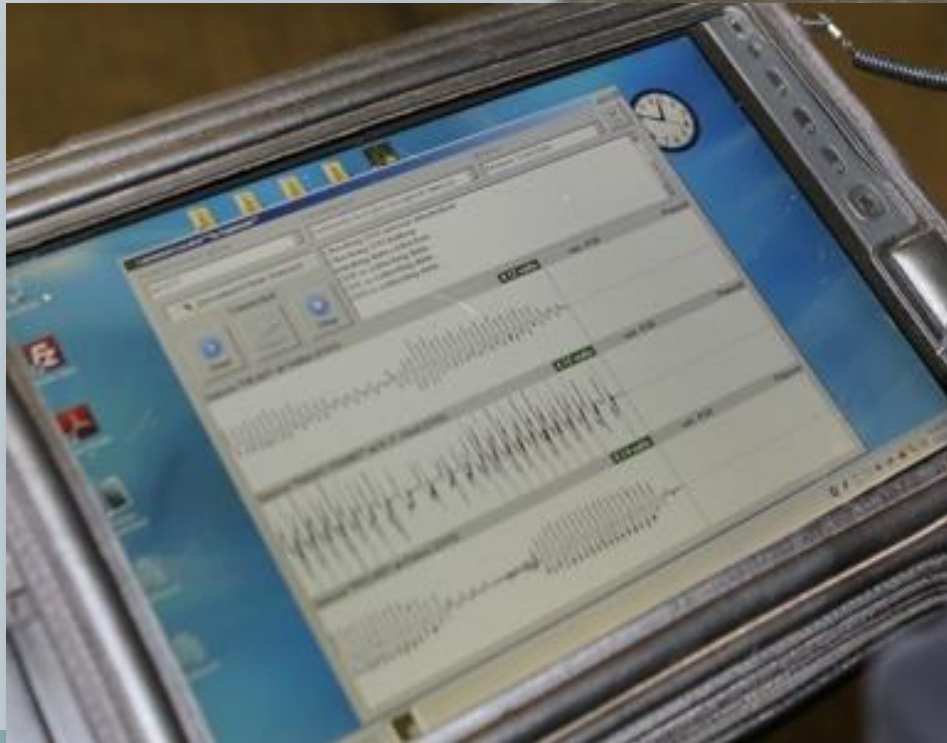
Some clinical cases were objectively studied (DJD)

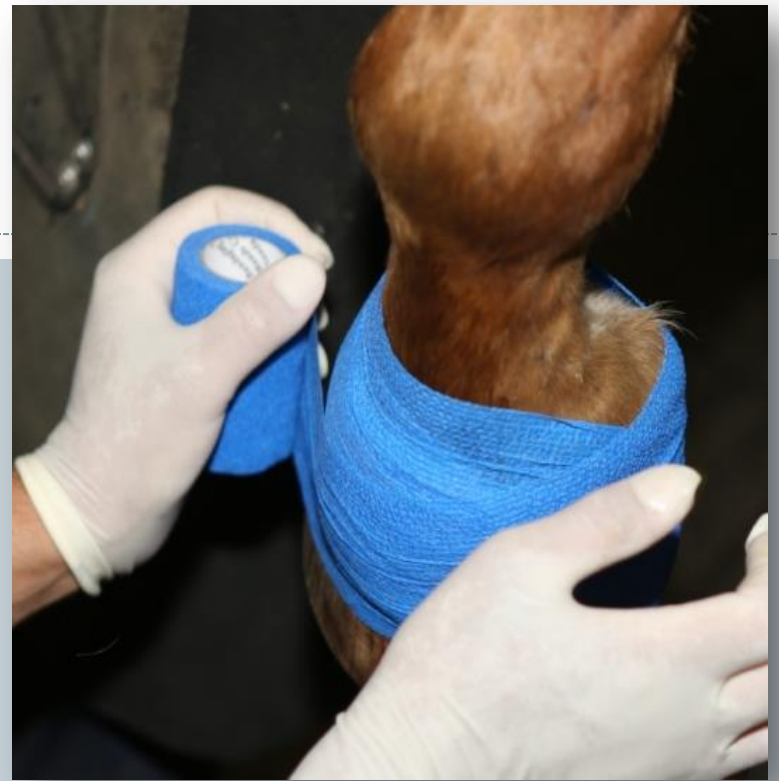
Objective tools to evaluate the efficiency and the chronology
of the clinical effects

LAMENESS LOCATOR®

by EQUINOSIS 



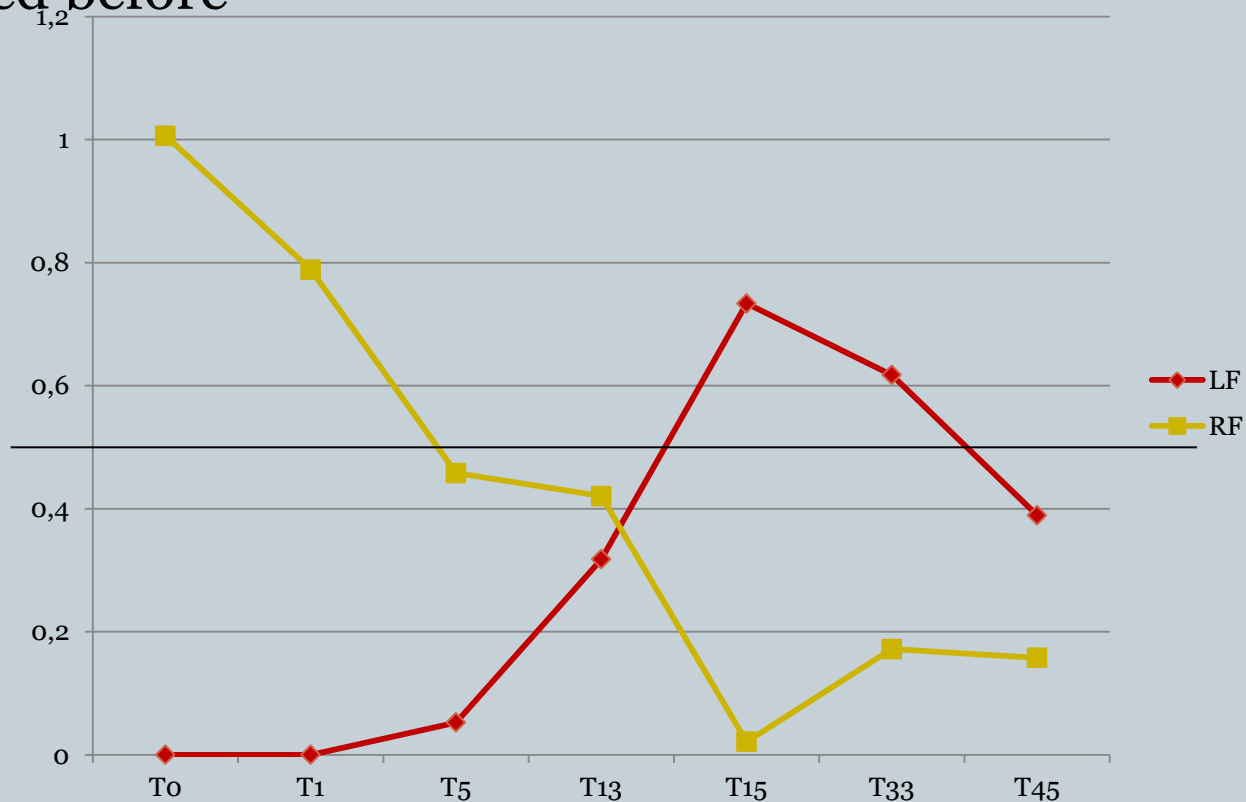




Clinical case 1



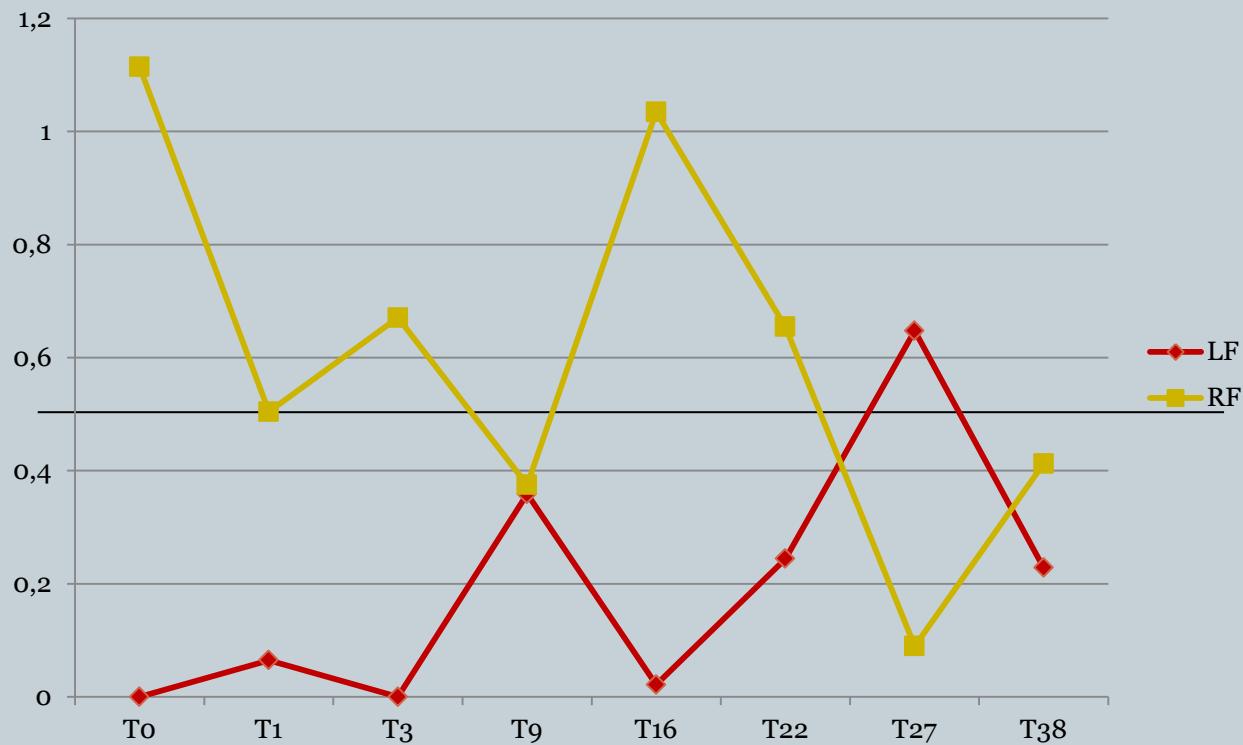
- Digital DJD in an old mare. Moderate lameness RF > LF
- Never treated before



No significant lameness during 5 months after injection

Clinical case 2

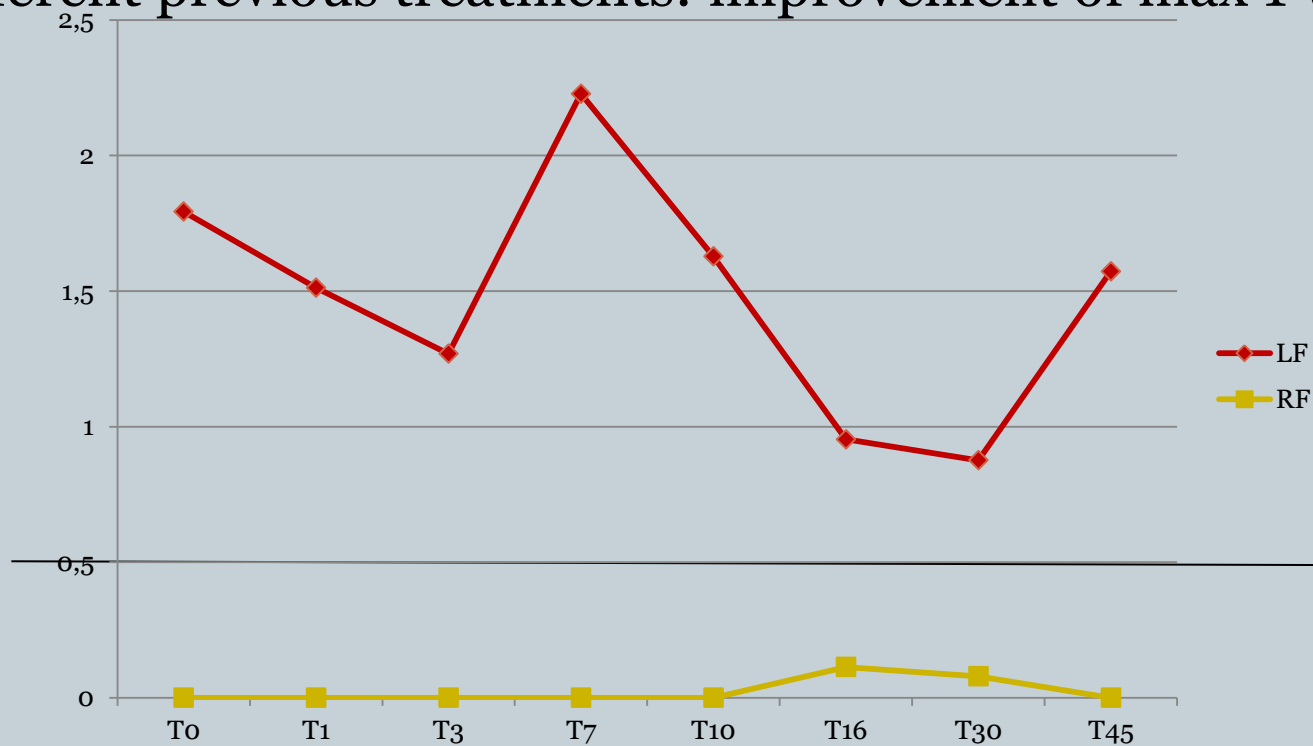
- Digital DJD in an old mare. Moderate lameness RF > LF
- Never treated before



No significant lameness during 6 months after injection

Clinical case 3

- Chronic and severe DJD in a mare LF > RF
- Different previous treatments: improvement of max 1 week



Improvement till T35

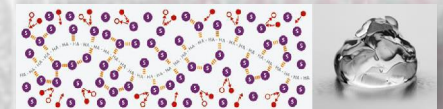
Clinical case 4



- A 12 years old Belgian sport horse gelding (3* eventing competitions). He showed a slight left forelimb lameness (grade II/V). Radiographically and echographically, we observed slight degenerative signs in the fetlock. Lameness resolved completely with an intra-articular analgesia.
- The lameness was observable during the first week but decreased progressively. The horse was sound at 10 days after injection. The horse did some slight dressage exercise the first week and then increased progressively the intensity of its work. The lameness was thereafter undetectable during 2 months and he competed again.

Conclusion

- Efficacy similar to an association HA+corticoids
- No doping
- No adverse effect
- 1 injection eventually to be repeated in severe cases



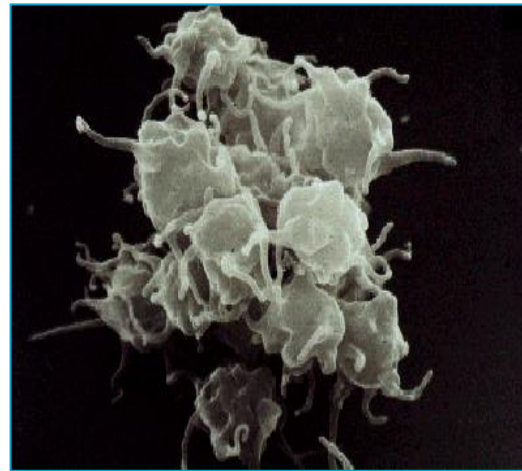


Growth factors

PrP Kits RegenVet^R



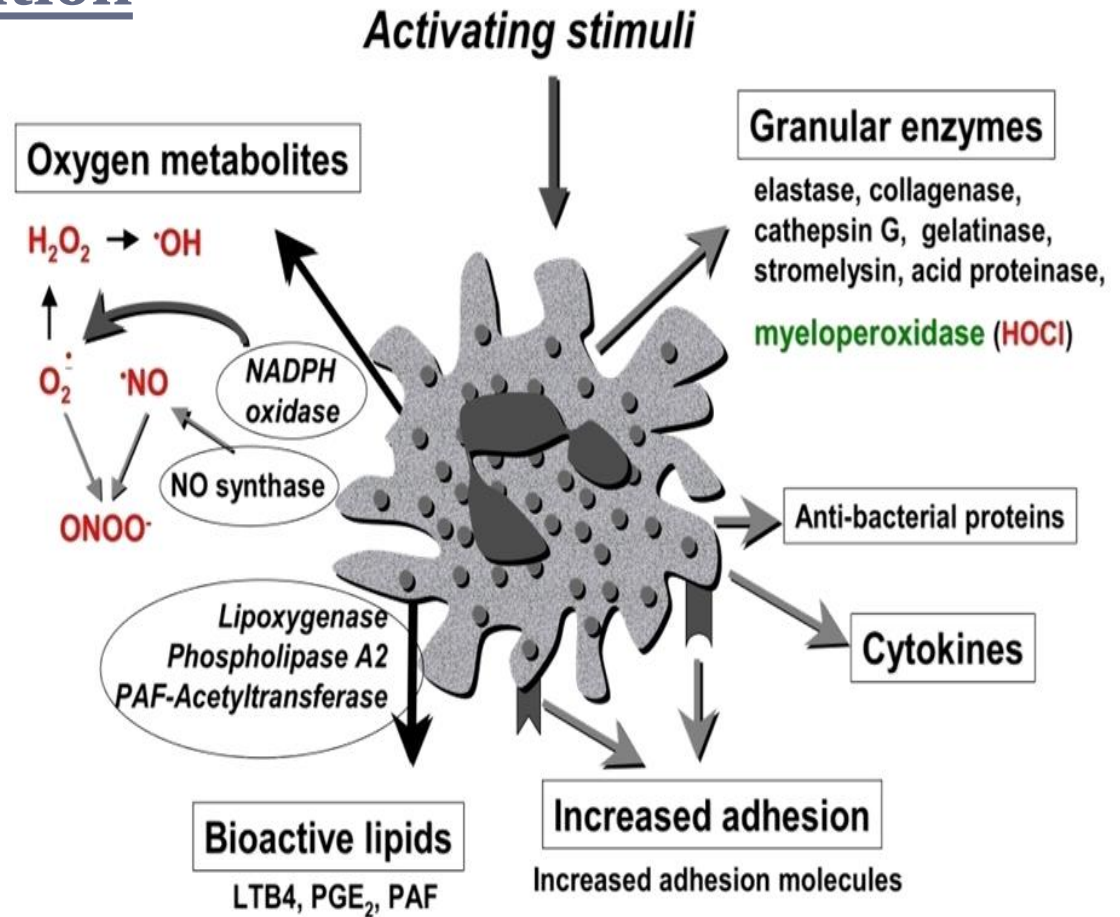
Platelet Rich Plasma: rich autologous source of many growth factors



Activation: via calcium or in situ

Point of attention: neutrophils activation with release of endogen pro-inflammatory cytokines → deleterious effects

Neutrophils activation



➡ Important to select a method that avoid the presence of neutrophils.
Home made techniques are often not validated.

PrP Kits RegenVet^R – Concept



- **Mechanism of action**

- Mononucleated blood cells have an immune-modulatory and anti-inflammatory effects
- Platelets that have been activated by tissue contact or by the addition of calcium will release growth factors which are necessary for optimal tissue healing

- **Indications**

Locomotor system

- Tendinopathy and desmopathy
- Premature degenerative arthropathy

Ophthalmology

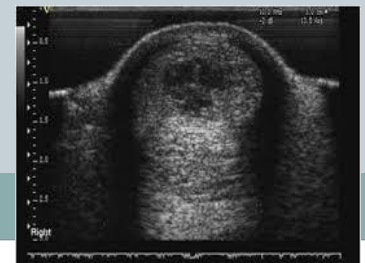
- Corneal ulcers
- Lacerations and ocular trauma



- **Advantages of validated kits**



- Specific tubes allowing the elimination of neutrophils and erythrocytes during the centrifugation process
- Elevated concentration of platelets (4 to 6 times plasmatic concentration)
- Elevated concentration of growth factors (TGF β , PDGF, IL1 ra)
- Low concentration of pro-inflammatory cytokines (TNF α , IL1 β , IL6) and oxidative and proteolytic enzymes (no neutrophil activation)
- The procedure is entirely aseptic
- Possibility to add regenerative cells or hyaluronic acid
- Obtention of a coagulum after activation used in localised lesions (tendons, ligaments)



Intra-articular use of PRP

- **PRP alone**: innocuity, anabolic effects

Synovial fluid growth factor and cytokine concentrations after intra-articular injection of a platelet-rich product in horses.

Textor JA1, Willits NH, Tablin F. Vet J. 2013 Oct;198(1):217-23.

Synovial fluid growth factor and cytokine concentrations after intra-articular injection of a platelet-rich product in horses.

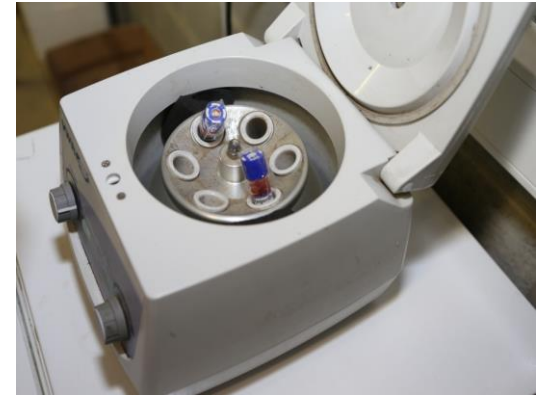
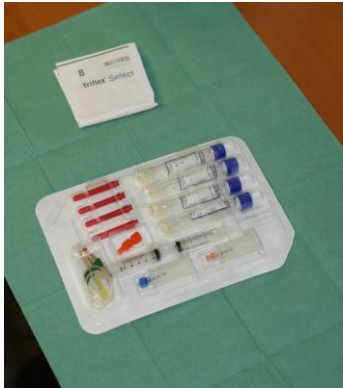
Textor JA1, Willits NH, Tablin F. Vet J. 2013 Oct;198(1):217-23.

- **PRP associated with HA**: synergy

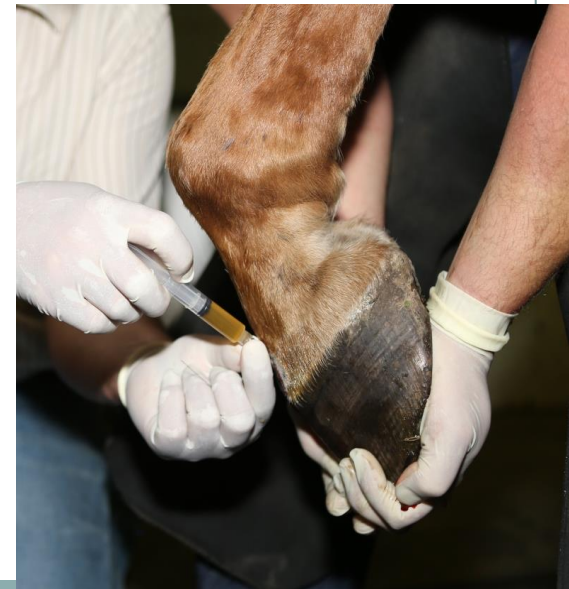
Synergistic anabolic actions of hyaluronic acid and platelet-rich plasma on cartilage regeneration in osteoarthritis therapy. Chen WH1, Lo WC2, Hsu WC1, Wei HJ1, Liu HY1, Lee CH3, Tina Chen SY1, Shieh YH4, Williams DF5, Deng WP6 Biomaterials. 2014 Dec;35(36):9599-607.

- **PRP + stem cells**: theoretical interest

Kits and sampling



Procedure





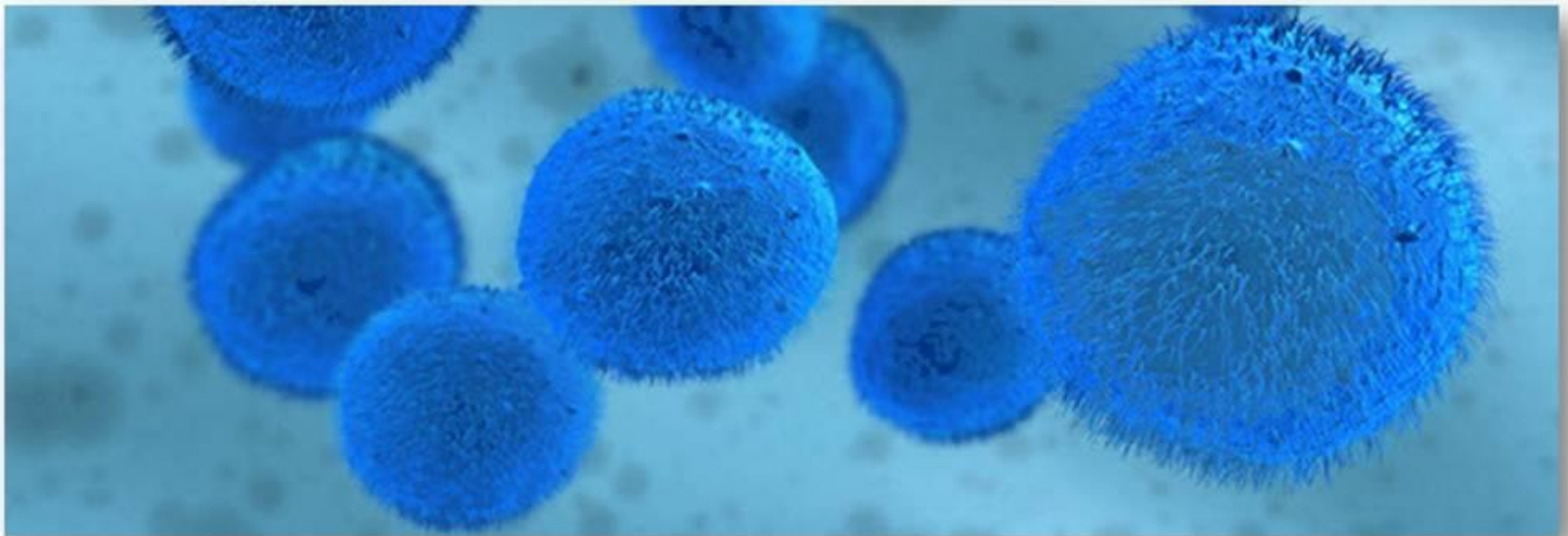
Stem cells

Origins

Mechanism of action

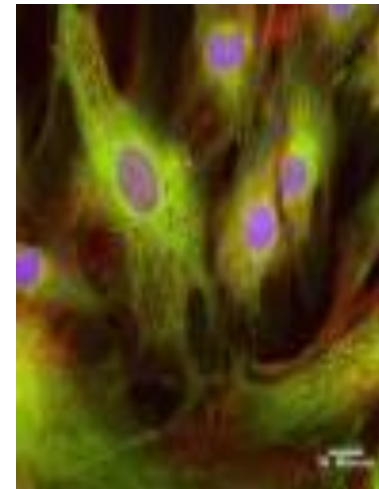
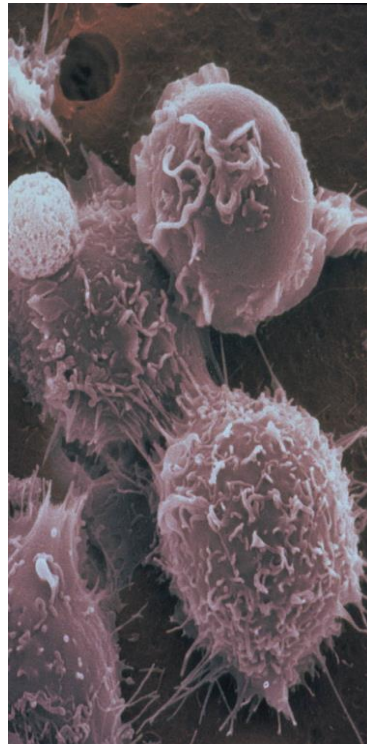
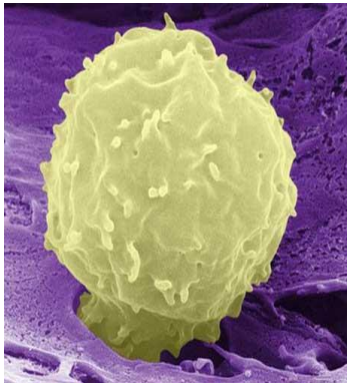


Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells



Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells.

Mesenchymal stem cells are undifferentiated cells originating from the mesoderm, an embryonic connective tissue. In adult horses, we found them mostly in bone marrow or in adipose tissue.



The majority of organs and tissues contain a small part of stem cells:
intestine, liver, pancreas, brain, muscles, tendons, cartilage, cornea, ...

Sources of stem cells for therapeutic use:

- Embryo (➔ risk of teratoma formation ?)
- In adult horses:
 - Bone marrow
 - Blood
 - Fat tissue,
 - Umbilical cord and placenta
 - and MUSCLES
- Autologous vs allogeneic

Classical techniques of sampling



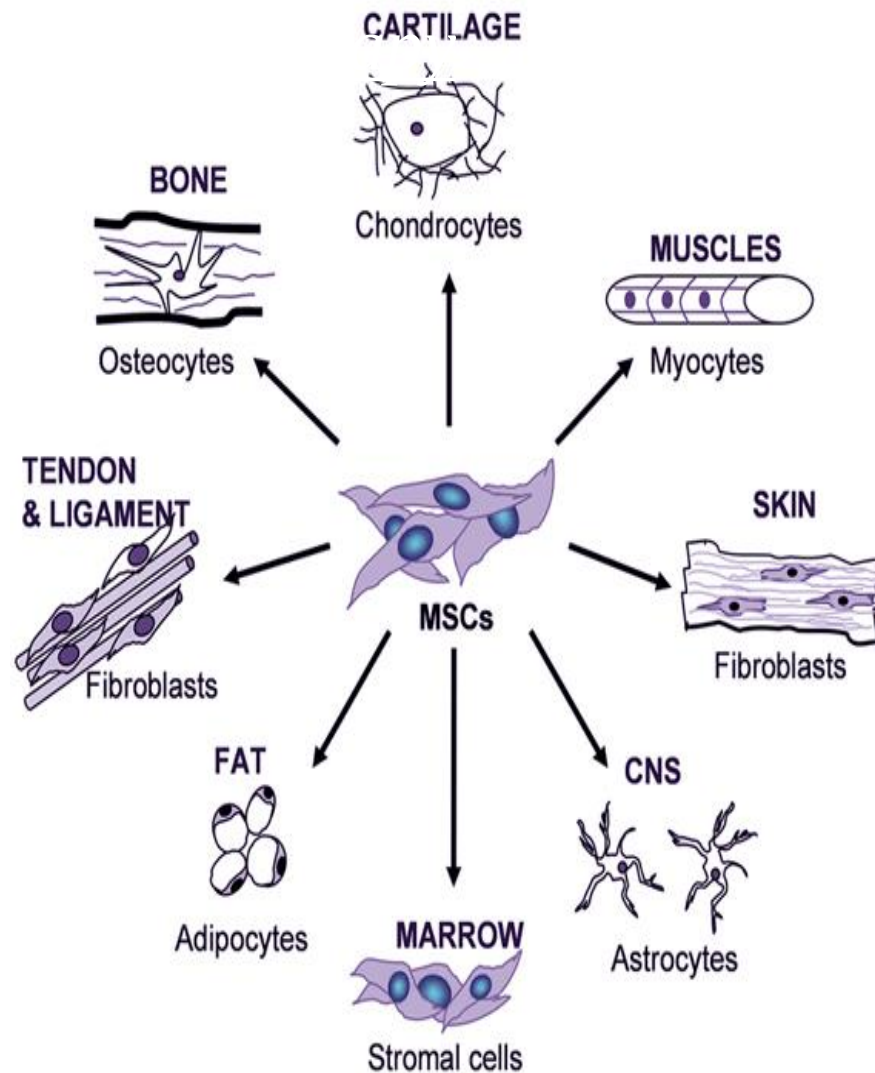
Bone marrow
(sternum)



Fat biopsy



Liposuction



Multi-lineage potential of adult mesenchymal stem cells Grassel *et al.* 2007

Mechanisms of action of stem cells

- Differentiation and engraftment
- But first, paracrine effects
 - decrease the production of pro-inflammatory cytokines (TNFa, IL-6, IFN-g),
 - enhance the production of anti-inflammatory cytokines (IL10, IL20, ...) by the white blood cells
 - inhibit the T- and B-lymphocytes T et B, dendritic cells → immunomodulation (ease the engraftment)
 - promote angiogenesis → nutrient intake at the level of the lesion
 - release of antiapoptotic factors → enhance the repair of the lesion
- Supply of defective cells
- Mitochondrial transfer

Therapeutic use of allogenic stem cells

- Interesting concerning the paracrine effect and the immunomodulation (before differentiation)
- 2 papers mention a **clinical efficacy** in tendon and articular diseases with stem cells originating from blood or umbilical cord **but in association with PRP**
 - Clinical follow-up of horses treated with allogeneic equine mesenchymal stem cells derived from umbilical cord blood for different tendon and ligament disorders. Van Loon VJ1, Scheffer CJ, Genn HJ, Hoogendoorn AC, Greve JW. Vet Q. 2014;34(2):92-7
 - Regenerative therapies for equine degenerative joint disease: a preliminary study. Broeckx S1, Zimmerman M2, Crocetti S3, Suls M2, Mariën T4, Ferguson SJ5, Chiers K6, Duchateau L7, Franco-Obregón A8, Wuertz K5, Spaas JH1. PLoS One. 2014 Jan 20;9(1):e85917.
- **BUT: What about differentiation?**
 - Equine bone marrow-derived mesenchymal stromal cells are heterogeneous in MHC class II expression and capable of **inciting an immune response** in vitro. Schnabel LV, Pezzanite LM, Antczak DF, Felipe MJ, Fortier LA. Stem Cell Res Ther. 2014 Jan 24;5(1):13.

Therapeutic use of autologous stem cells

Fat tissue (biopsy or liposuction): 3 possibilities

- Extemporaneous preparation: **Stromal vascular fraction** which is a rich source of preadipocytes, mesenchymal stem cells (MSC), endothelial progenitor cell and some other types of cells

Advantages: immediately available (90 min), some recognized efficacy in tendonitis

- Possibility to send the sample to the lab and receive in 48h the extracted stem cells without culture but without quality control.
- Possibility to send the sample for extraction, culture and cryopreservation of stem cells

Effect of autologous adipose tissue-derived mesenchymal stem cells on neovascularization of artificial equine tendon lesions. Conze P1, van Schie HT, Weeren Rv, Staszky C, Conrad S, Skutella T, Hopster K, Rohn K, Stadler P, Geburek F. Regen Med. 2014 Nov;9(6):743-57.

Therapeutic use of autologous stem cells

Bone marrow derived stem cells

Recognized efficacy demonstrated in tendinitis and desmitis but in association with PRP

Autologous bone marrow mesenchymal stromal cells for regeneration of injured equine ligaments and tendons: a clinical report. Renzi S1, Riccò S, Dotti S, Sesso L, Grolli S, Cornali M, Carlin S, Patruno M, Cinotti S, Ferrari M. Res Vet Sci. 2013 Aug;95(1):272-7.

Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. Smith RK1, Werling NJ, Dakin SG, Alam R, Goodship AE, Dudhia PLoS One. 2013 Sep 25;8(9):e75697.

Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. Godwin EE1, Young NJ, Dudhia J, Beamish IC, Smith RK. Equine Vet J. 2012 Jan;44(1):25-32.

Recognized efficacy demonstrated in osteoarthritis, microfracture but in association with PRP

Clinical outcome after intra-articular administration of bone marrow derived mesenchymal stem cells in 33 horses with stifle injury. Ferris DJ1, Frisbie DD, Kisiday JD, McIlwraith CW, Hague BA, Major MD, Schneider RK, Zubrod CJ, Kawcak CE, Goodrich LR. Vet Surg. 2014 Mar;43(3):255-65.

Evaluation of intra-articular mesenchymal stem cells to augment healing of microfractured chondral defects. McIlwraith CW, Frisbie DD, Rodkey WG, Kisiday JD, Werpy NM, Kawcak CE, Steadman JR. Arthroscopy. 2011 Nov;27(11):1552-61

Application areas of cell therapy

- Locomotory disorders
- Ophthalmology
- Wound healing
- Respiratory system
- Reproduction
- Laminitis
- Systemic autoimmune disease
- Septic shock

Routes of administration

- Intralesional
- Intra-articular, intra-synovial
- Intravenous
- intravenous regional perfusion
- Intra-arterial

Scintigraphic comparison of intra-arterial injection and distal intravenous regional limb perfusion for administration of mesenchymal stem cells to the equine foot. Trela JM1, Spriet M, Padgett KA, Galuppo LD, Vaughan B, Vidal MA. Equine Vet J. 2014 Jul;46(4):479-83.

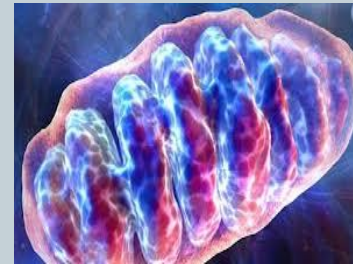
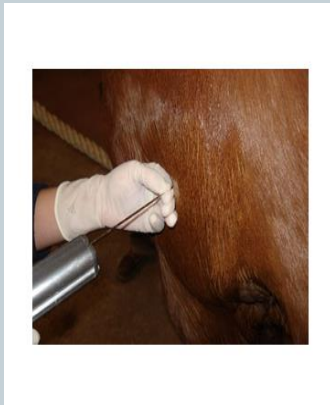


Muscular derived stem cells



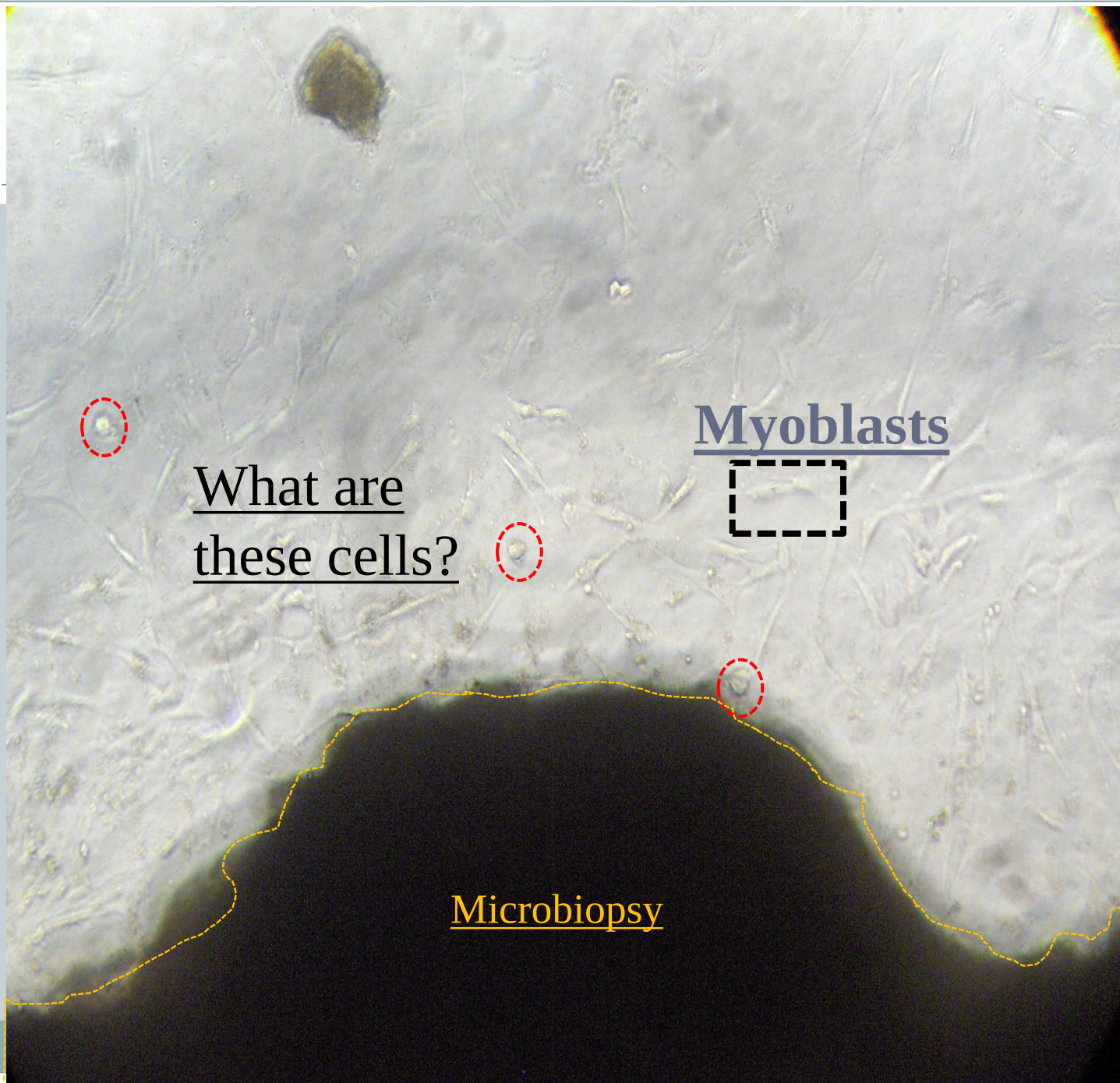


- 2008-2012 : PhD thesis – Justine Ceusters
 - Culture of muscular cells coming from microbiopsy
 - In order to study mitochondrial function



- We found 2 different types of cells ???





What are
these cells?

Myoblasts

Microbiopsy



MDSC's (muscle-derived stem cells) or
SMSC's (skeletal muscle stem cells)

We had:

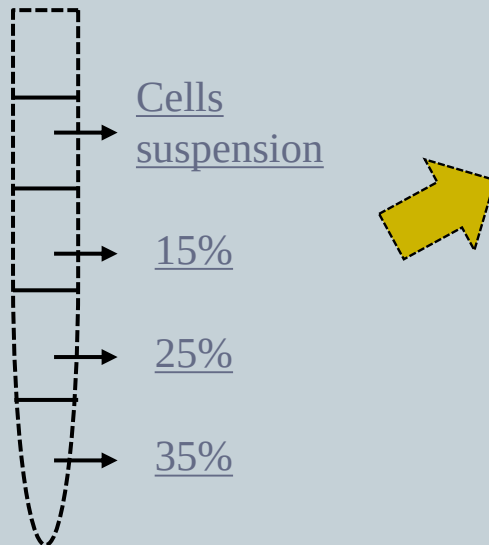
- To select and culture those cells specifically
- To verify the pluripotent nature of the cells
- To check their innocuity and potential efficacy



Selection of the stem cells

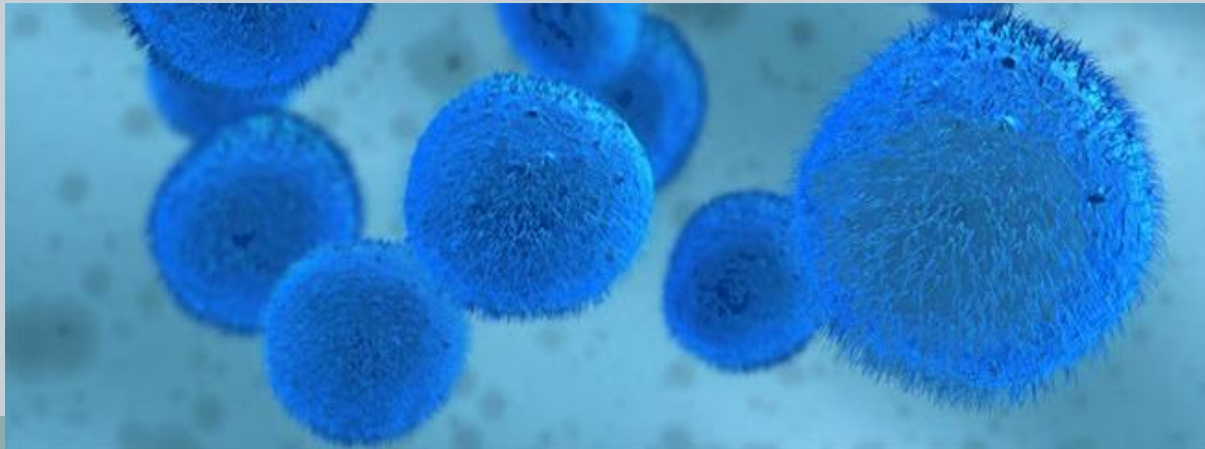


Centrifugation, selection of a specific fraction and culture





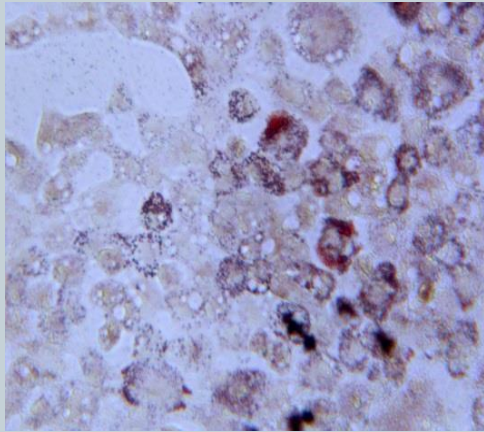
- CFU assay (ability to proliferate)
- Growth
- Immunophenotyping
- Differentiation



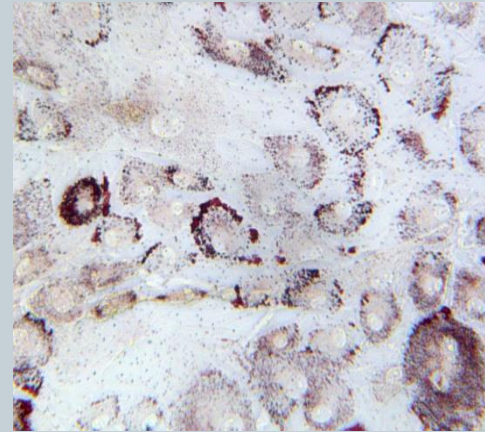
Differentiation



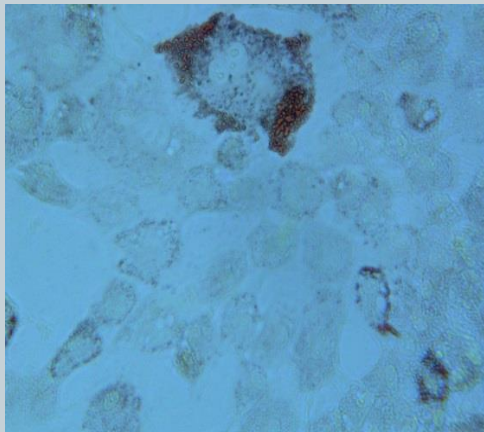
- Adipogenic



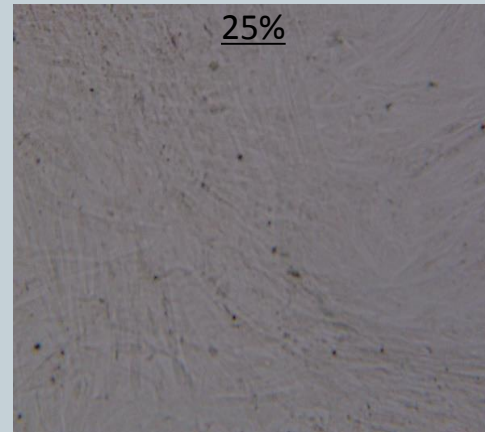
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15-
25%



25-
35%

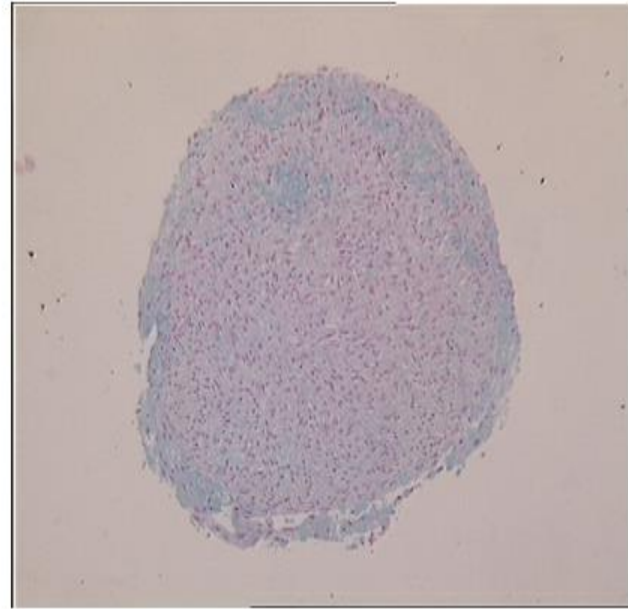
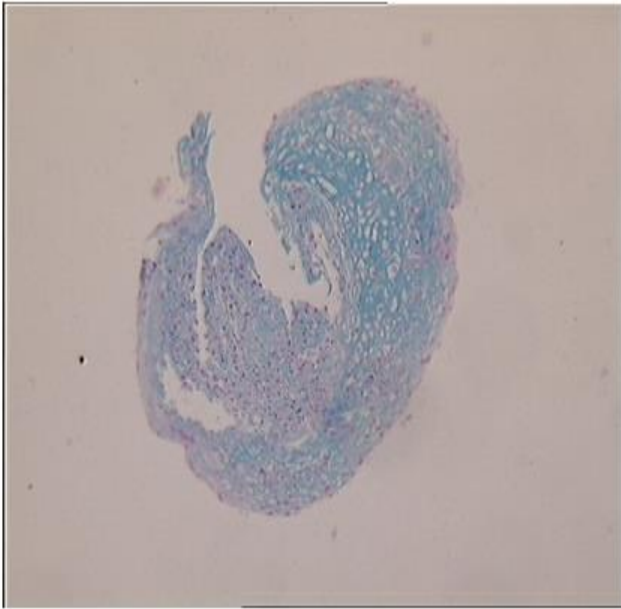


Control

Differentiation



- Chondrogenic



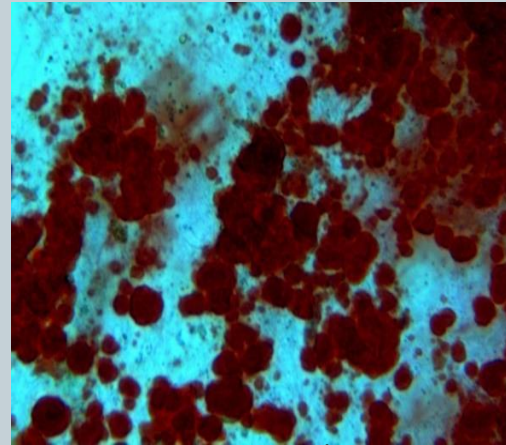
Differentiation



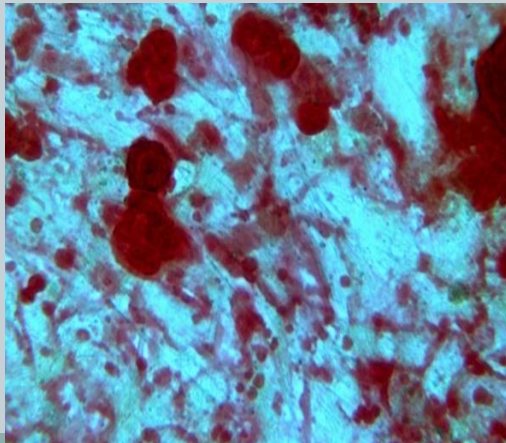
- Osteogenic



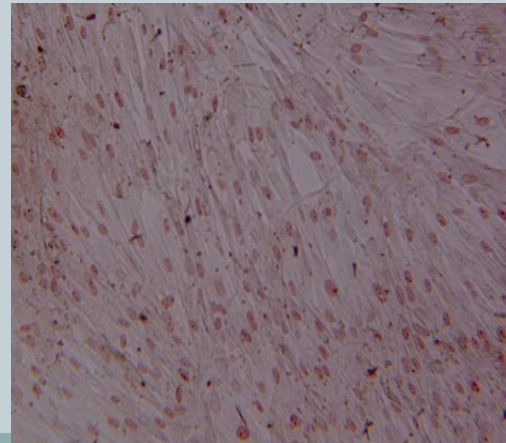
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15-25%



25-35%



Control

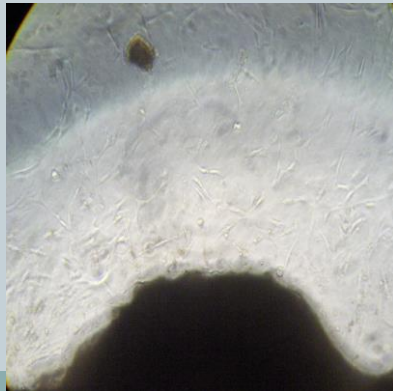
Innocuity and efficacy

52

- Autologous treatment
- Injected in joints of healthy horses
- Used in therapy of tendonitis and desmitis
- No adverse reaction and good results in the repair of tendons
- Decision to create Revatis
 - Office and lab
 - Clean room
 - Quality controlled procedures



From research to ... you



Production

20.10⁶ MdSC
+/- 5 weeks post micro biopsy

Quality control



Microbiological analysis

- No contamination
- Results available in 1 week



- Either the cells are available for therapeutic injection
- and/or we can cryopreserve the cells

Prevention ... Biobank



Available if needed



For example:
Suspensory ligament desmitis in
endurance horse...

➔ Treating soon ➔ better prognosis





**Stem cells innovation:
the future of your horse**



**Conserve
the cells of your horse
in the RevaTis Biobank**

www.revatis.com

Jumping
Ambassador

Endurance
Ambassador?

A close-up profile shot of a white horse with a dark mane and tail, looking over a wooden fence. The background is a blurred landscape with bare trees.

Abouftamal en janvier 2014

Thank you for your attention