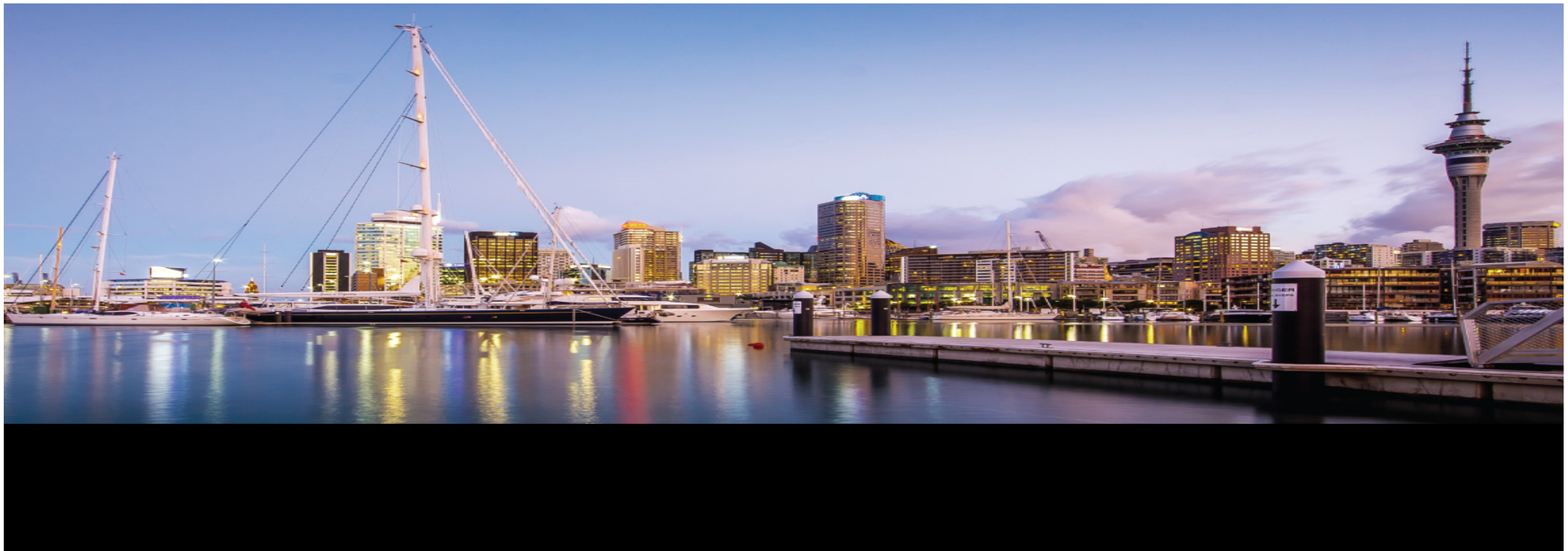
Conflict of Interest Declaration

Royalties & stock options – NONE

Consulting Income – NONE

Research & Education Support:
NonVasiv GmbH., TRT LLC., MediSpec Ltd., & Delsys Inc. USA.

Other support - NONE

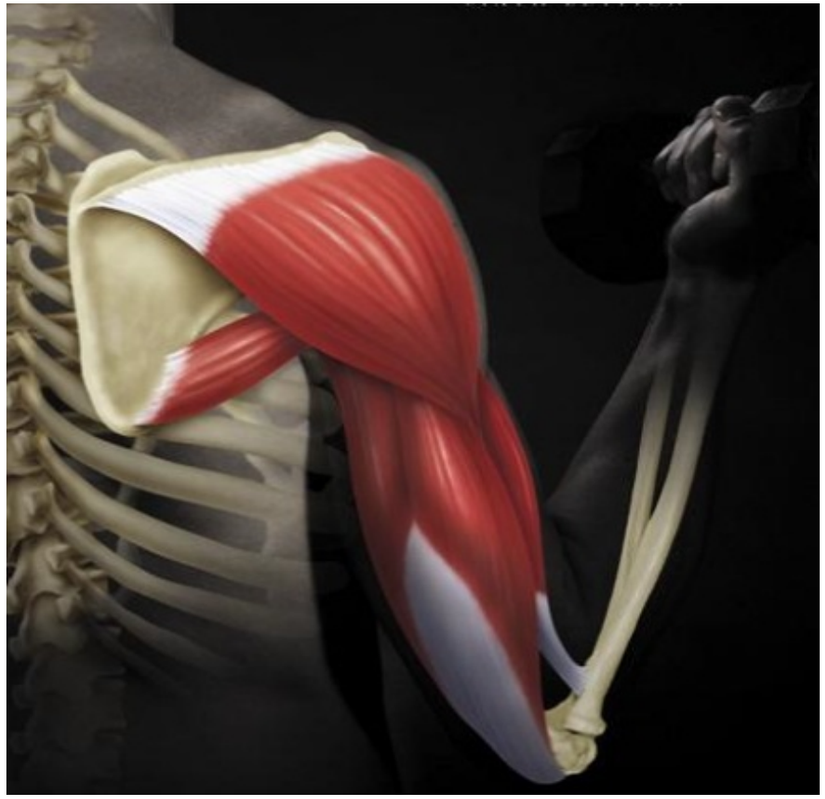


AIM

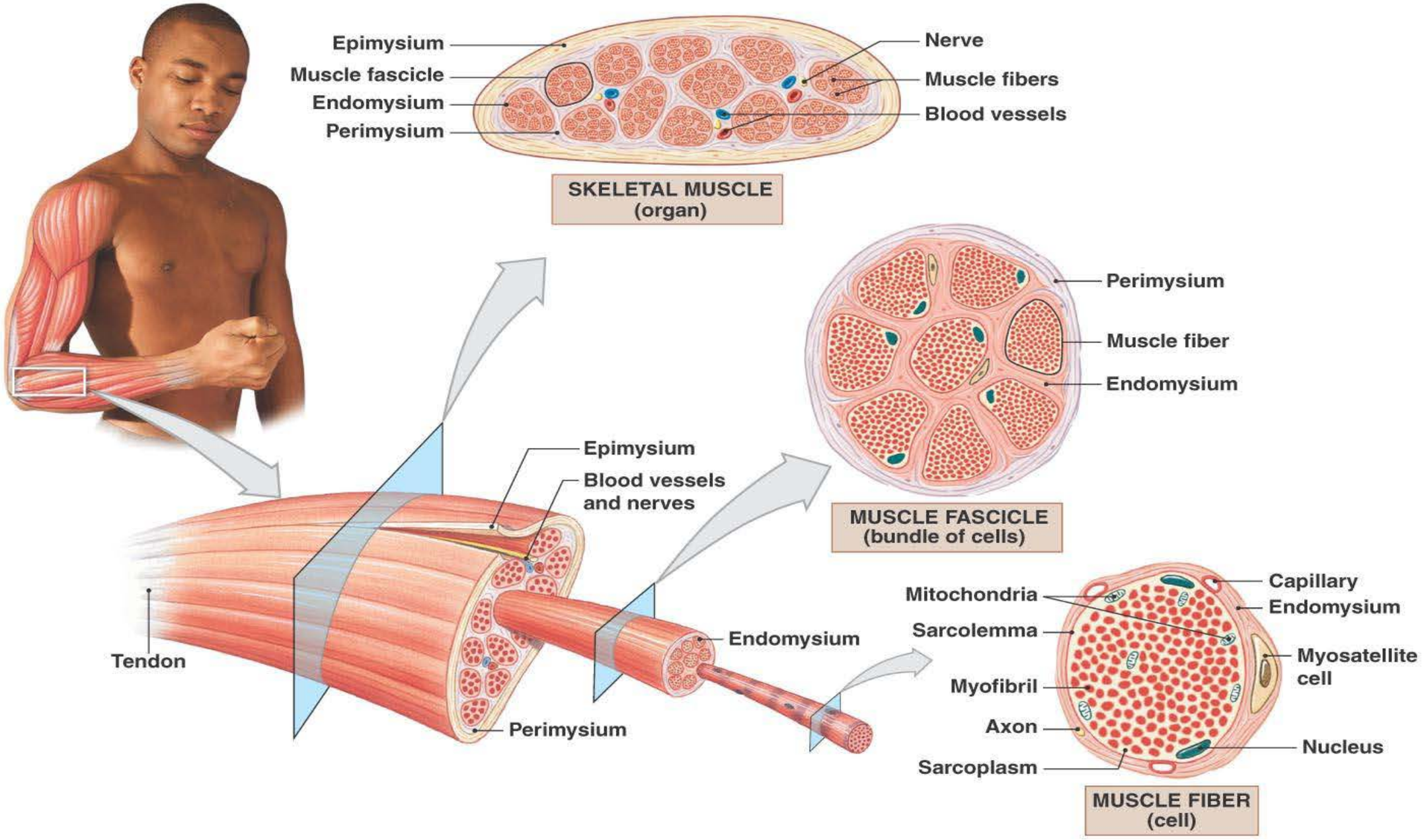
To promote investigation & collaboration in this area

SM: Introduction

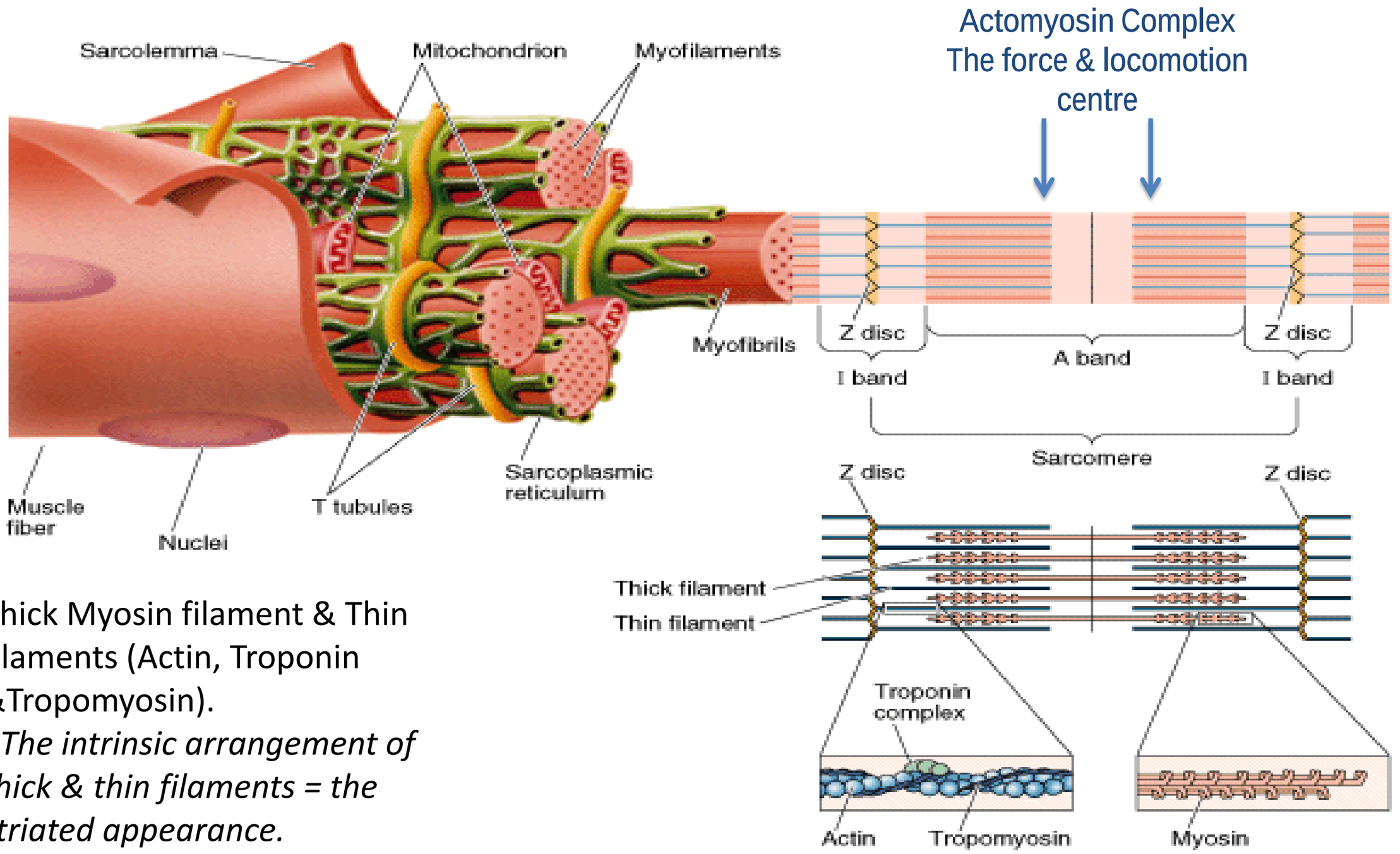
- Controlled via voluntary somatic muscle control
- Striated
- Approx. >400 SM in body
  - Muscle fibers, fascia, nerves & blood vessels
- 45% - 50% of body weight
- 70% - 75% water content
- Approx. 50% of total body protein content
- Multiplex functionality
  - Respiratory facilitation
  - Energy storage
  - Power generation
  - Thermoregulation
  - Posture & locomotion
  - Auto-para-endocrine networking
  - Metabolic regulation
  - High plasticity



SM: Structural Organization



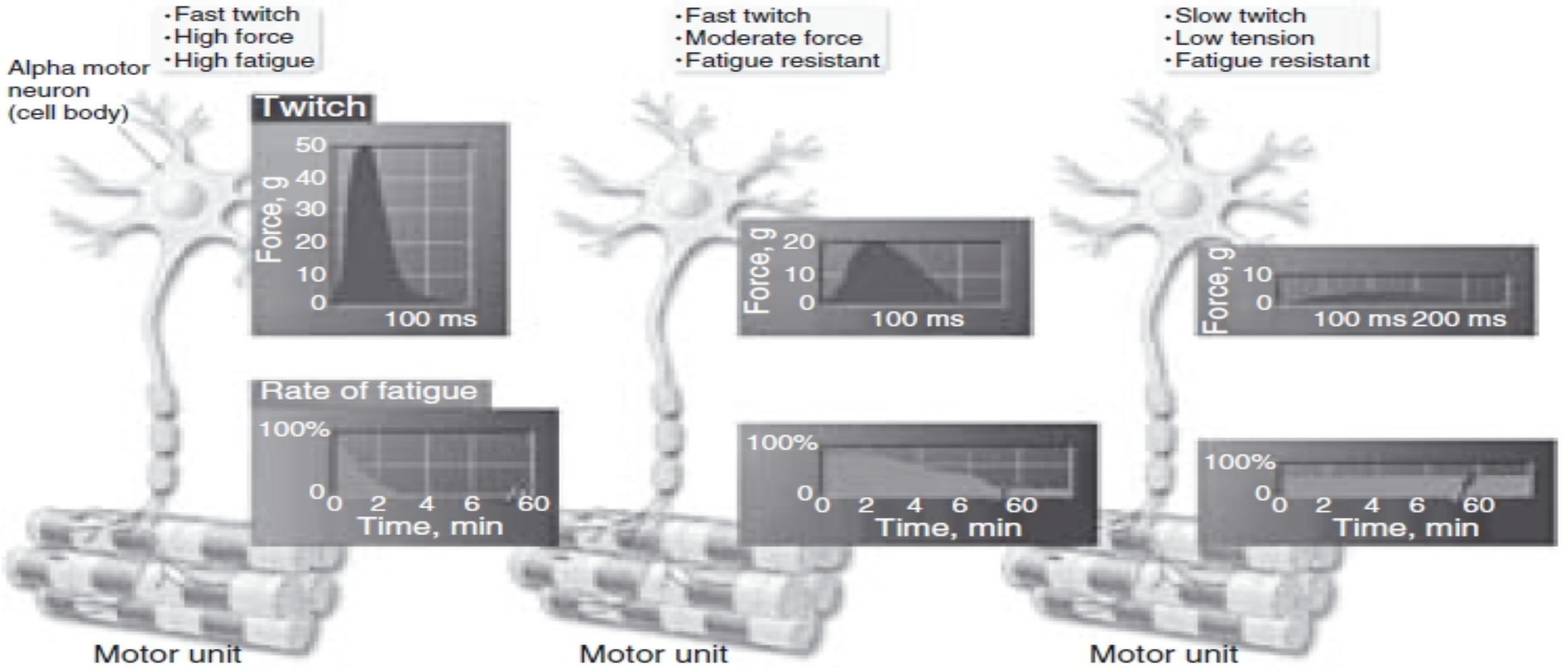
► Organization of a Muscle Fiber



Thick Myosin filament & Thin filaments (Actin, Troponin &Tropomyosin).

**The intrinsic arrangement of thick & thin filaments = the striated appearance.*

SM Fibre Types: Contraction Velocity, Force & Fatigue



Contraction Speed: 40-90ms
V Large Motor Neuron
Low Mitochondria
High Glycolytic & ATPase

Contraction Speed: 50-100ms
Large Motor Neuron
Moderate Mitochondria
High Glycolytic & Int. ATPase

Contraction Speed: 90-140ms
Small Motor Neuron
High Mitochondria
Low Glycolytic & ATPase

SM: Fibre Type

Muscle fibre type can be measured in 3 ways: myosin ATPase histochemistry, immunohistochemistry & metabolic enzymes (less commonly utilised).

1. Myosin ATPase staining
   - Staining intensities differ in pH sensitivity from each fibre type.
2. Immunohistochemistry
   - Variations exist between the fibre types on the basis of the different myosin heavy chain isoforms.
   - The MHC isoforms serves as ATPase conversion sites with varying ATP hydrolysis speed.
   - The main 3 isoforms are: MHCI; MHCIIa & MHCIIx
3. Metabolic enzyme
   - Provides information into metabolic pathways, describing muscle fibres as being aerobic / oxidative, fast-twitch oxidative, and slow-twitch oxidative.

SM: Fibre Type of Different Muscles

Hip Extensors

- hamstrings & G max. mixture of type I & II fibres (slightly > TI)

Plantarflexors

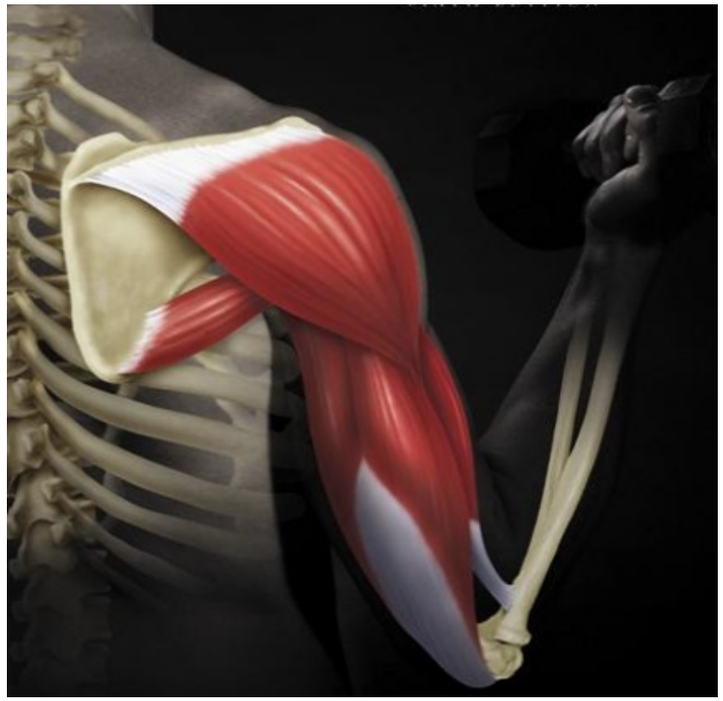
- soleus markedly TI.
- Gastrocs: mixture of TI & II

Knee extensors

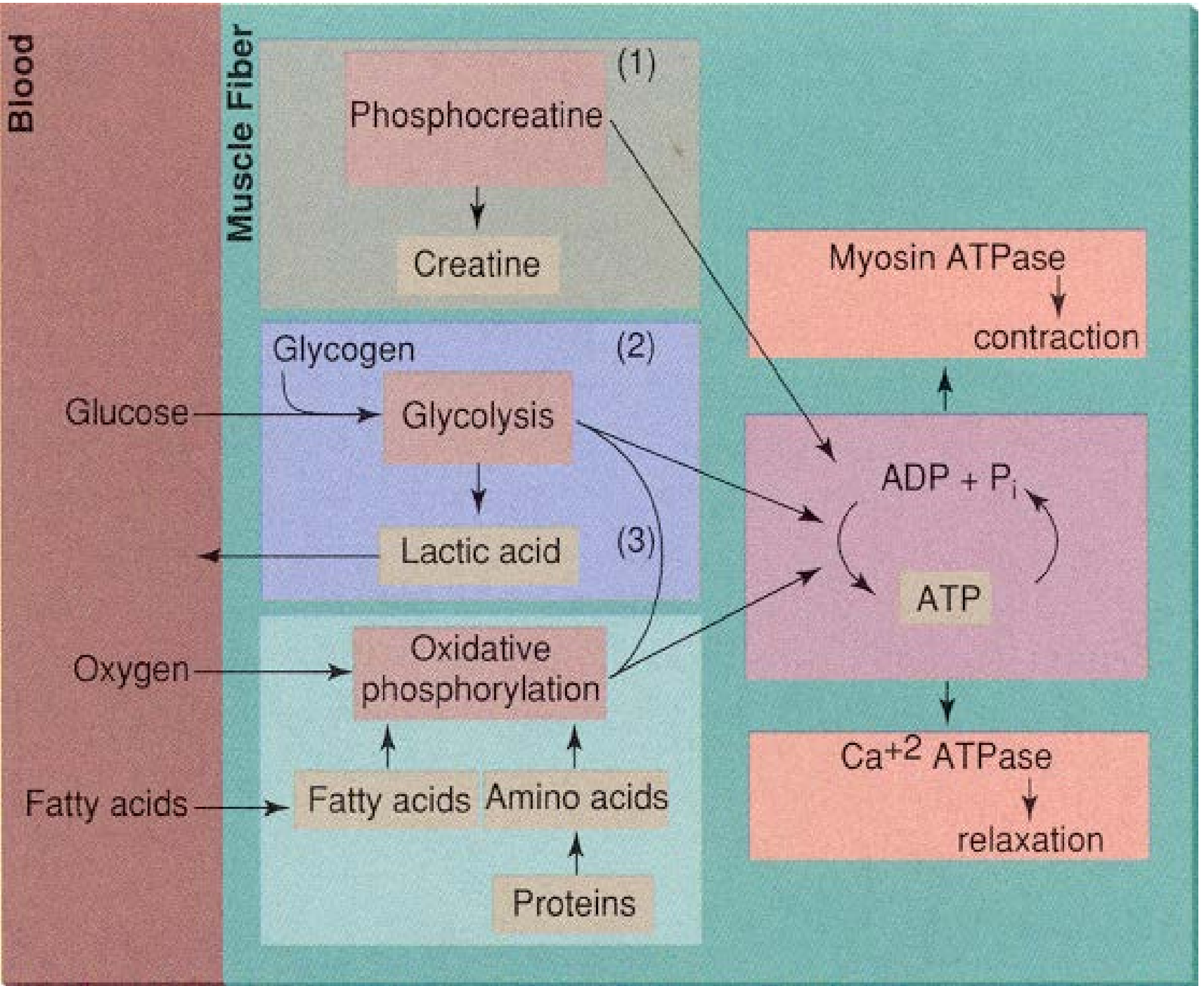
- rectus fem mixture (TI & TII; >II).

Shoulders:

- biceps, triceps & pectoralis (TII)
- Lat Dorsi (balanced TI & TII)



Sources of ATP



ATP Generation occurs in:

- Muscles
  - muscular contraction
- Cytoplasm
  - glycolysis
- Predominantly Mitochondria
  - oxidative phosphorylation (OXPHOS)

SM: Regeneration

Fundamentally 3 sequential overlapping stages:

- Inflammatory response
- Activation, differentiation & fusion of satellite cells
- Maturation and remodelling of new myofibres

SM: Functional Return

Muscle cells need:

1. Intracellular energy reserves (glycogen, creatine phosphokinase)
2. Optimal circulation (nutrient in; tissue waste disposal)
3. Normal O^2 levels
4. Normal pH
5. Lactic acid disposal & conversion (glucose)
6. Proteostasis / biogenesis (cellular folding; transcription; degradation & transport).
7. Satellite cell: functional niche signalling & activation

SM: Remodelling

Muscle fibre protein transcription, a complex process:

- Transcriptional catabolic & anabolic signalling (ie. miRNA; FOXO; mTOR; MYOG; Pax activity)
- Autophagy (eg. elimination of defective organelles for energy prod.)
- Hormonal signalling (ie. IGF1; SMAD's; Leptin)
- ATP conversion into cAMP (GPCRs; SM-Dopamine receptor 1 & 5 activation)
- Mechanical transcription & regulation
  - sacromeric-hubs ie Z-disk & M-lines
  - stretch / strain biomechanical responses onto matrix (satellite nice / zone)
- Circulation
- Tissue age / plasticity
- Satellite cell activation (a pivotal function of SM regeneration)

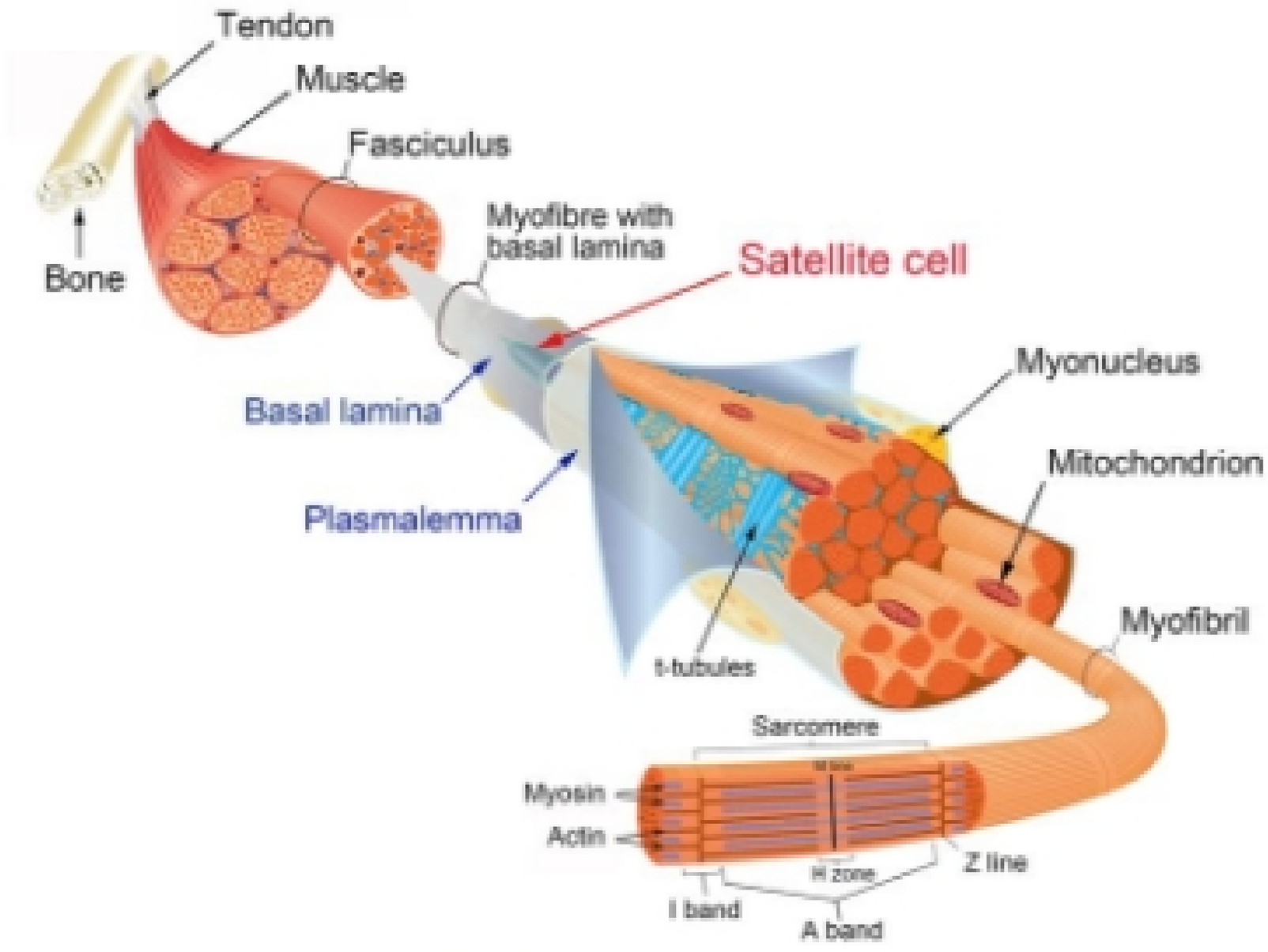
SM Satellite Cells

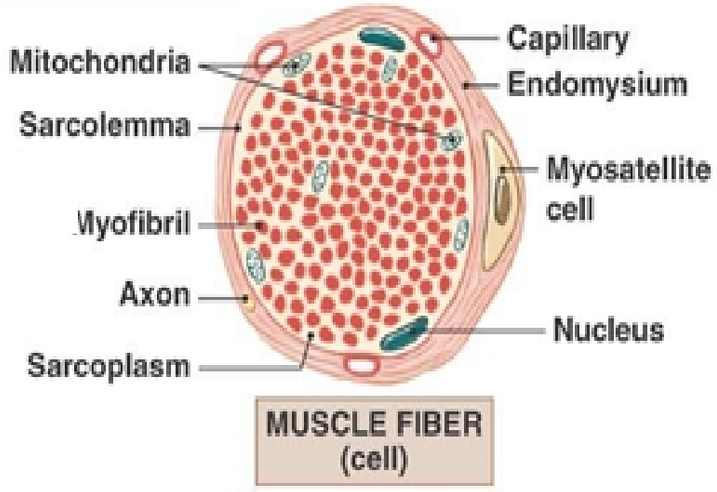
- Discovered by Alexander Mauro (over half century ago), and named as such due to their sublaminar location & intimate association with plasma membrane myofibers
- Quiescent state SC are activated by a mitogen due to injury.
- Proliferation & differentiation of SC during regeneration influenced by:
  - Innervation**
  - Vasculature
  - Hormones
  - Nutrition
  - Extent of injury
- Adult SMSC's self replicate & differentiate (into functional progeny), a bona fide stem cell. The process of self replication is governed by the structure and signalling in their niche / zone (ie Wnt signalling).

SM SC's: Identification markers

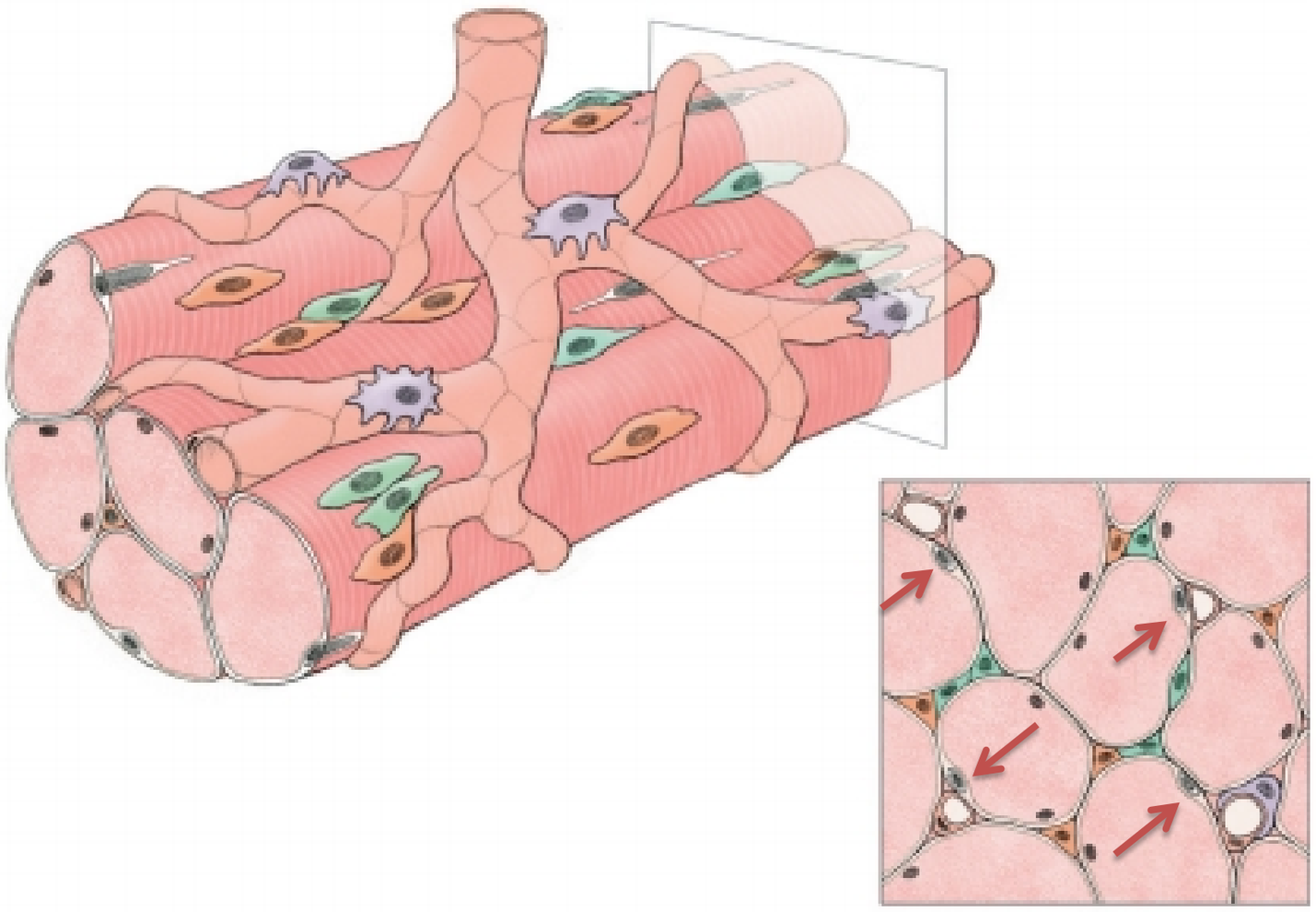
- SMSC's are classically identified based on anatomical location: just beneath the basal lamina, and outside the myofiber plasma membrane
- Most adult SMSC's express Pax7 (considered the canonical biomarker)
- Myogenic RF Myf5
- Homeobox TF Barx2
- Protein Cell adhesion molecule M-cadherin
- TRK c-Met
- Cell surface attachment receptor α7-integrin
- Transmembrane HSP sydecan-3 & 4
- Chemokine receptor CXCR4
- Caveolin-1
- Calcitonin receptor
- NEP lamin A/C
- Emerin

SM SC's: anatomical location

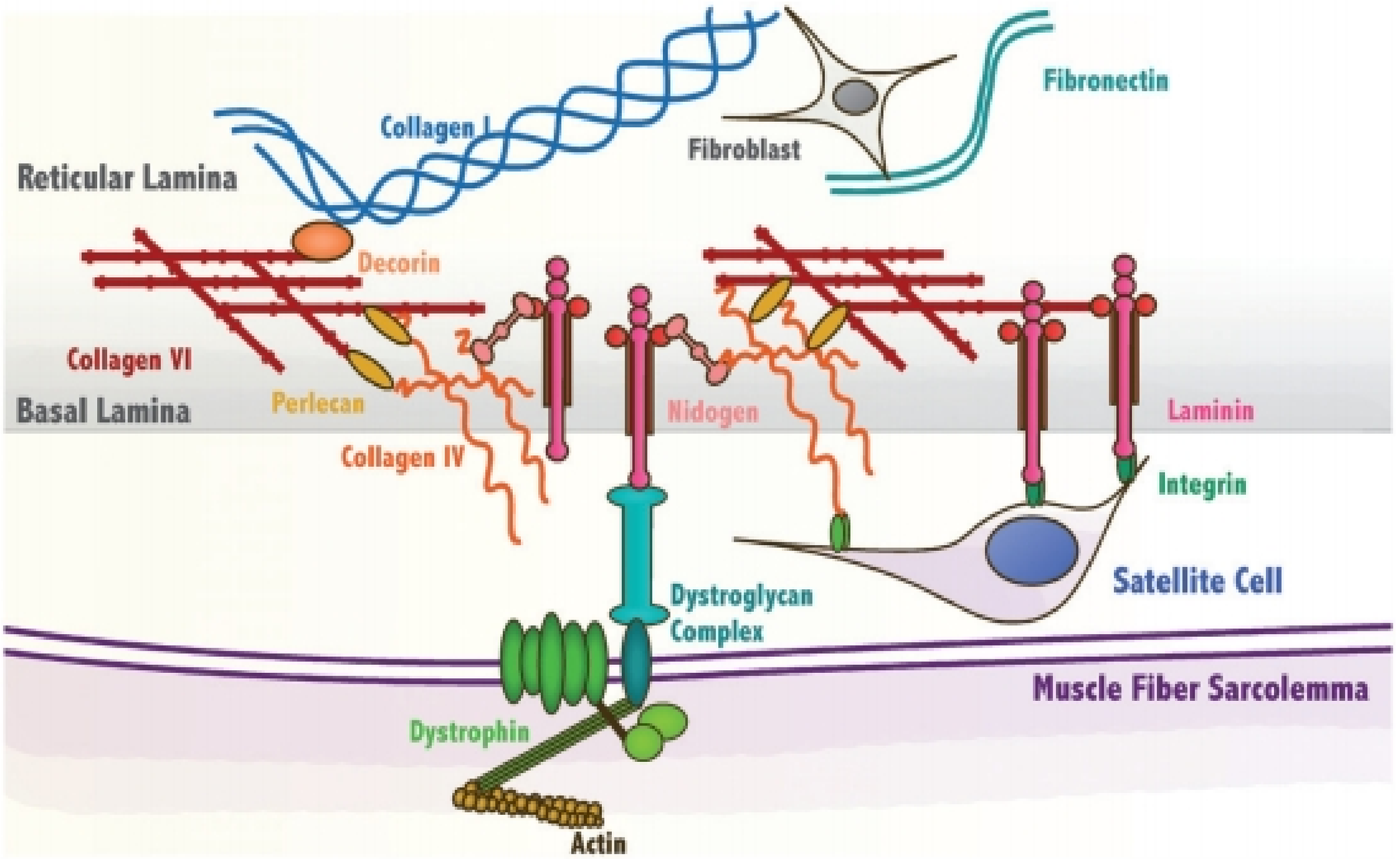




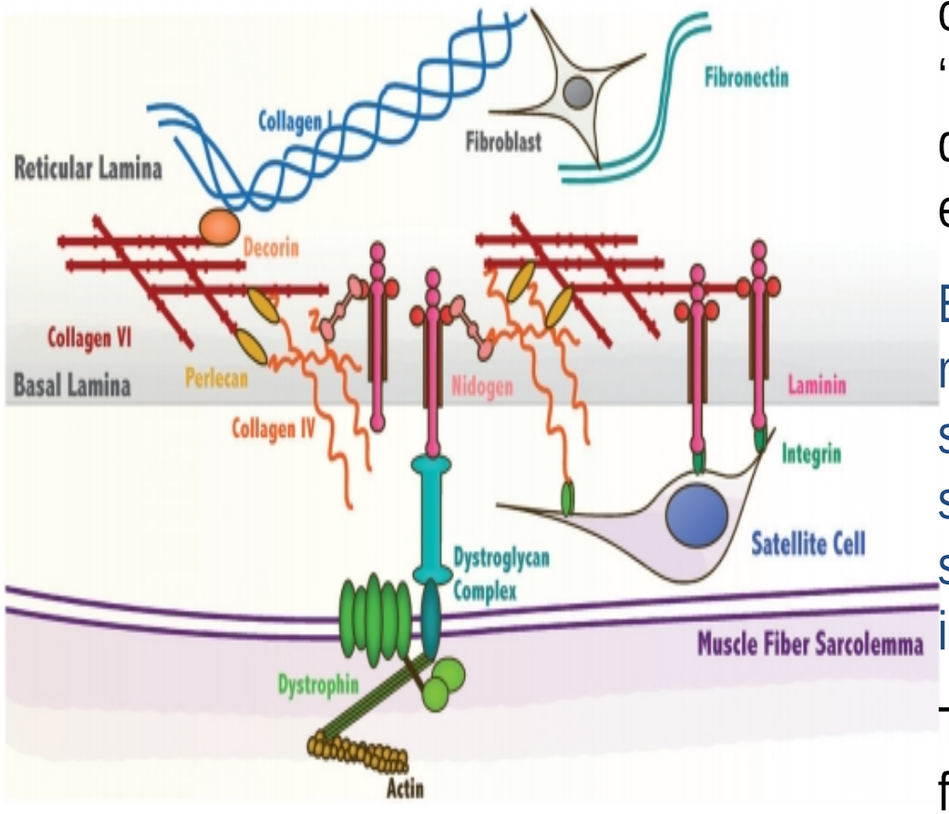
SM SC's: anatomical location



SM SC's: The Niche / Zone



SM SC's: The Niche / Zone

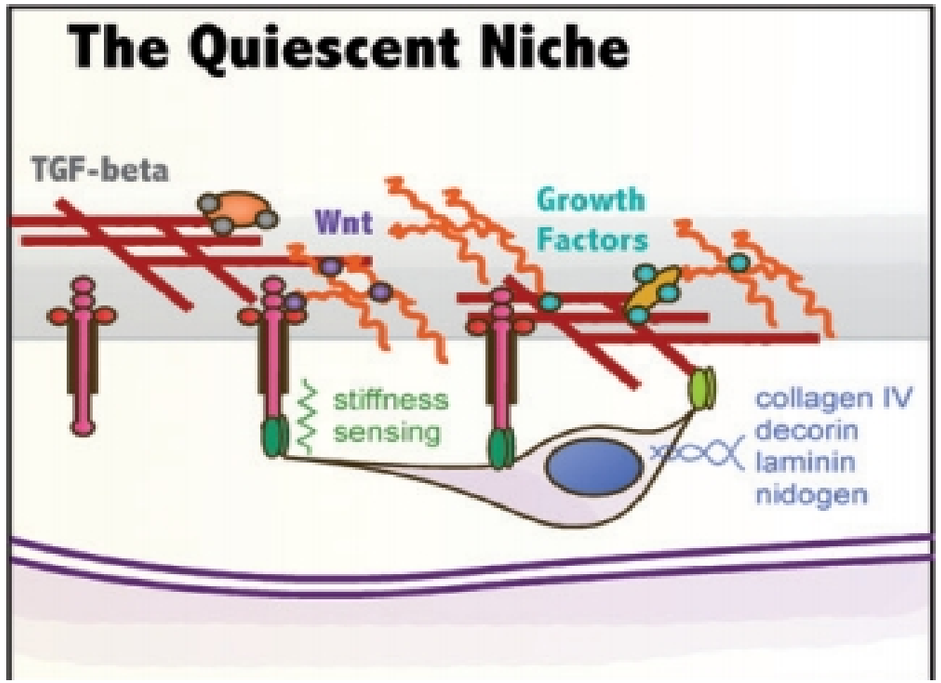


The 'Niche' is not merely an anatomical grid, but rather a dynamic communications conduit, sensing & transmitting signals (ie. biomechanical, chemical etc.) relaying the status & requirements of the tissue to its 'Regenerative Cell' source the Satellite Cells. Negative alterations or disruptions to the niche often result in defective regenerations in nearly every stem cell compartment of the region or body.

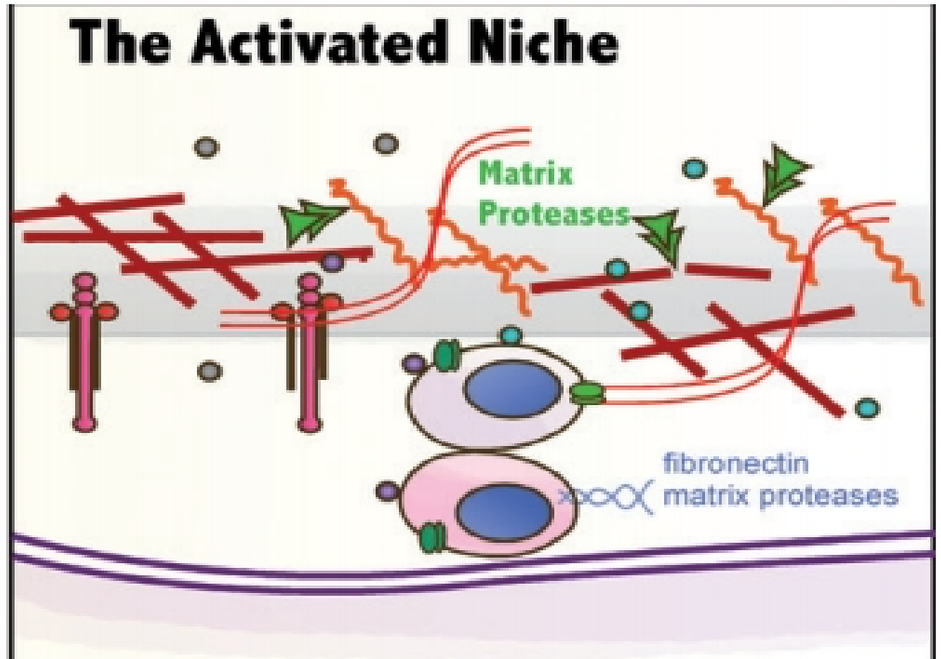
ECM components are considered essential mediators in the niche for the maintenance of stem cell identity, expression, and activation. It simultaneously provides the niche structural integrity, and physically separates the stem cell pool from other tissue resident cells. Stem cells sense & respond to the composition, porosity & stiffness of the ECM directly interacting with it via integrin focal adhesions.

The ECM surrounding muscle fibres comprise: collagens, laminins, fibronectin, glycosaminoglycans, short polysaccharide chains bound to core protein forming proteoglycans. This matrix termed 'Basement Membrane' is dual layered with the reticular lamina (superiorly) & basal laminar (inferiorly).

SM SC's: The Niche / Zone

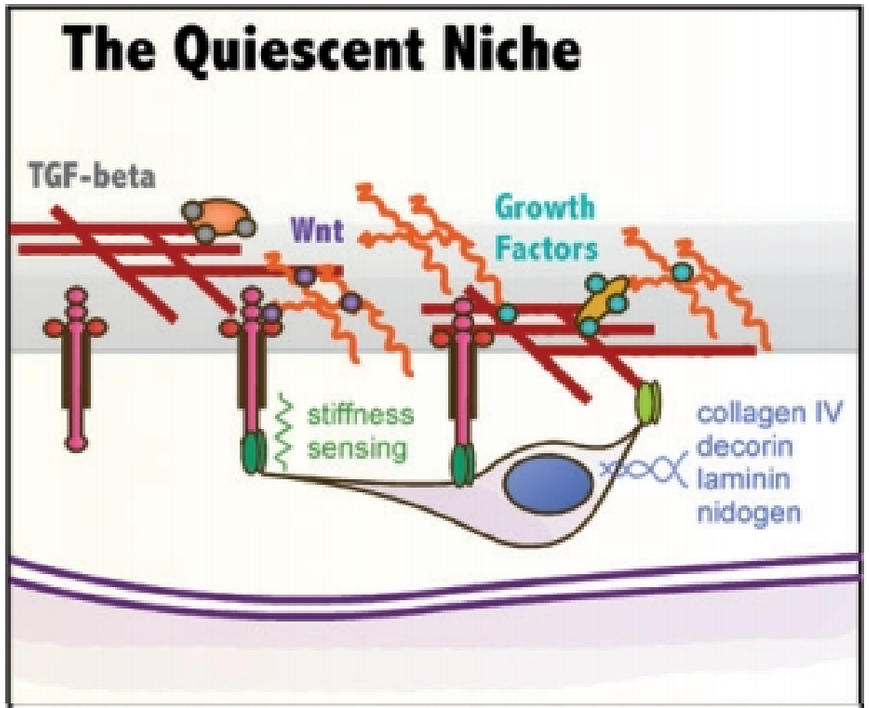


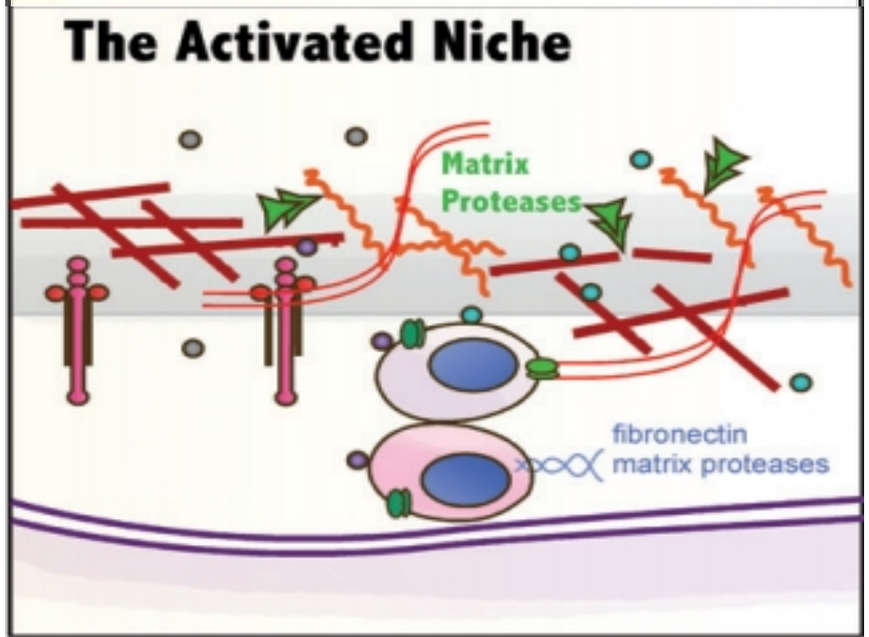
The quiescent SC senses the stiffness of its niche through integrins and expresses various matrix proteins to maintain its extracellular matrix (ECM). Within this matrix, growth factors and signalling molecules such as Wnts and TGF-b are sequestered, maintaining the ‘‘quiet’’ state.

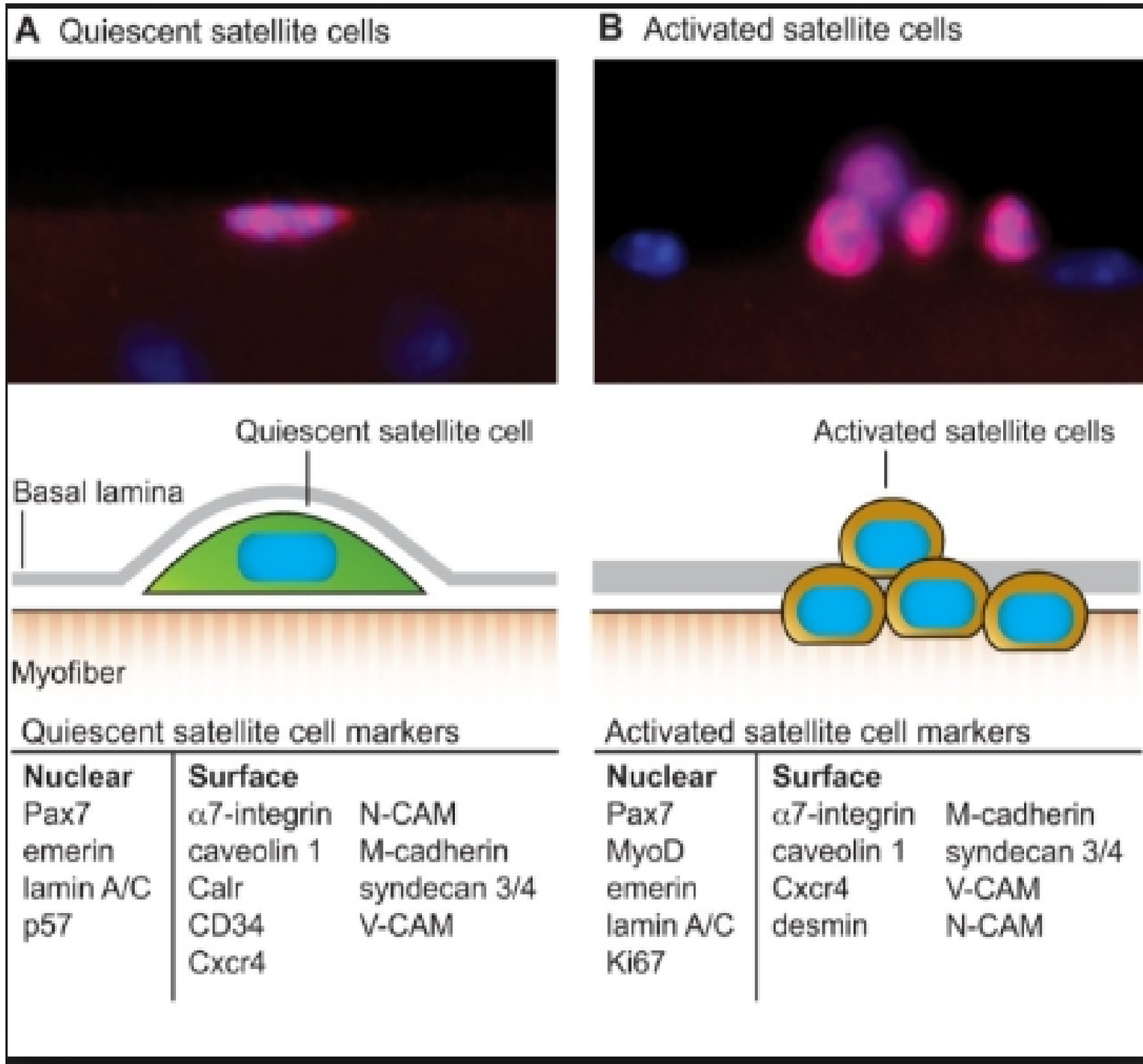


In response to injury or stimulus, components of the basal lamina are degraded by matrix proteases which results in the release of signalling molecules that play a role in activation and proliferation of the SC. The activated SC divides and some daughter cells begin to differentiate.

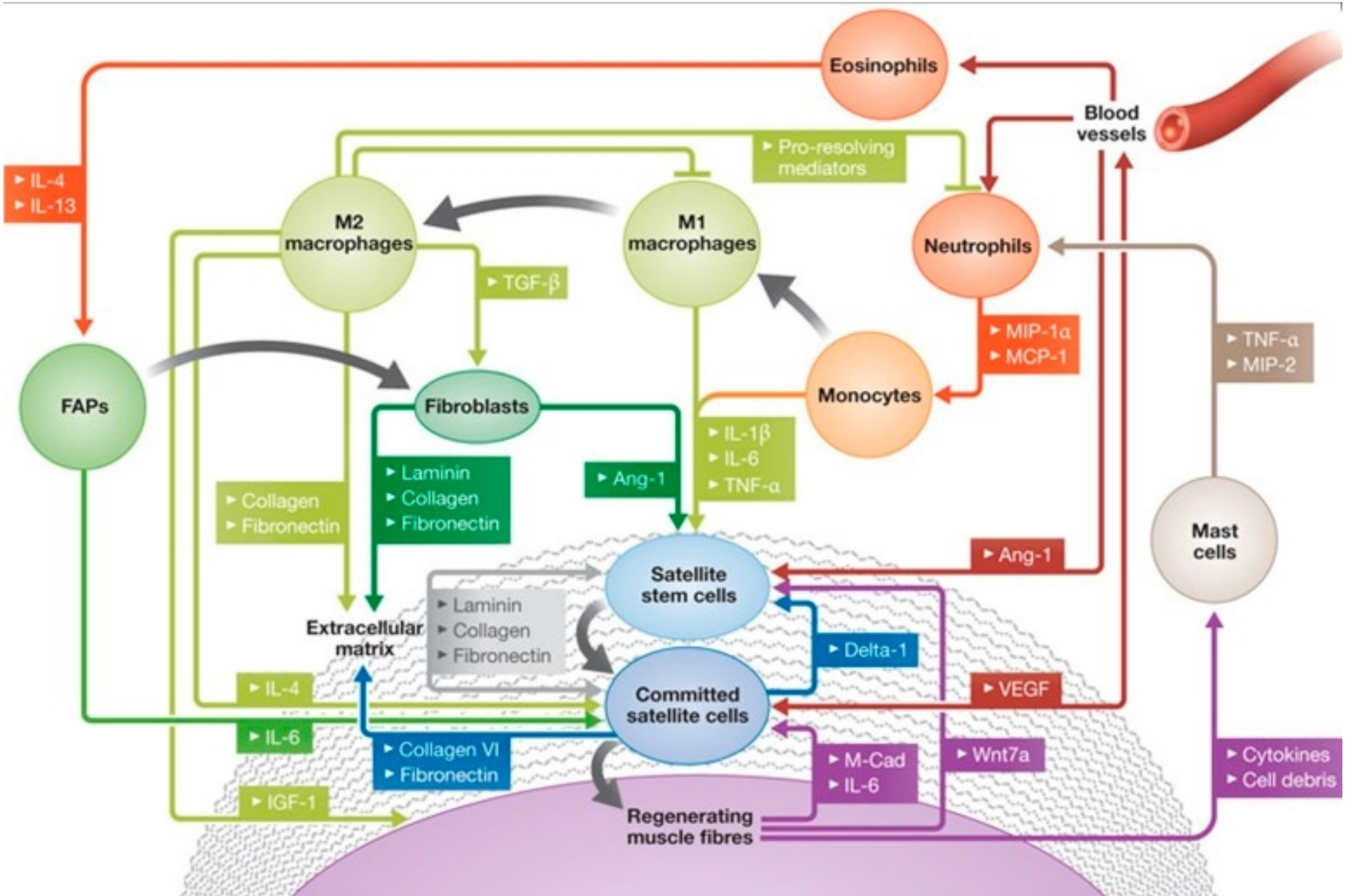
SM SC's: Expression & Activation Markers



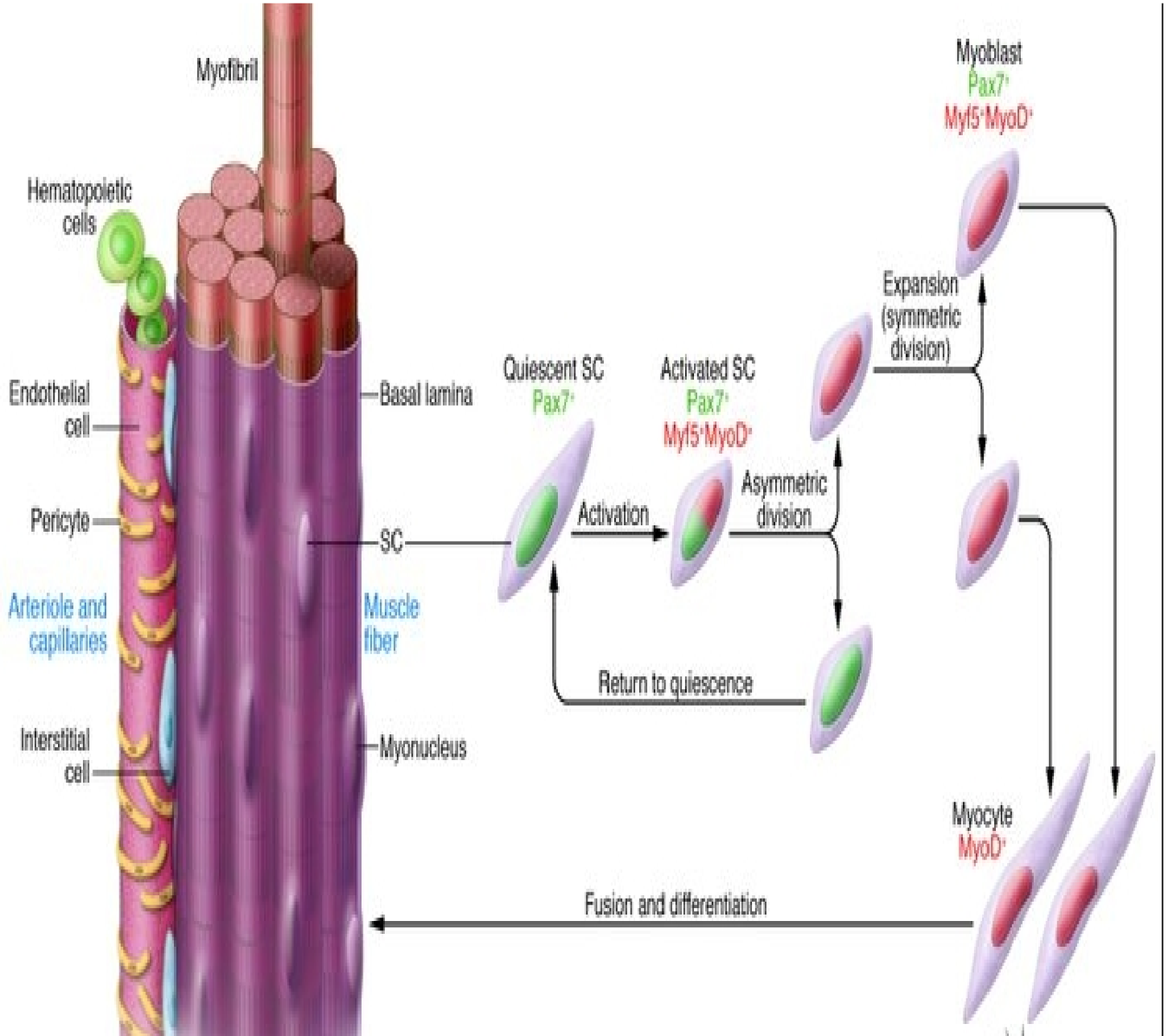


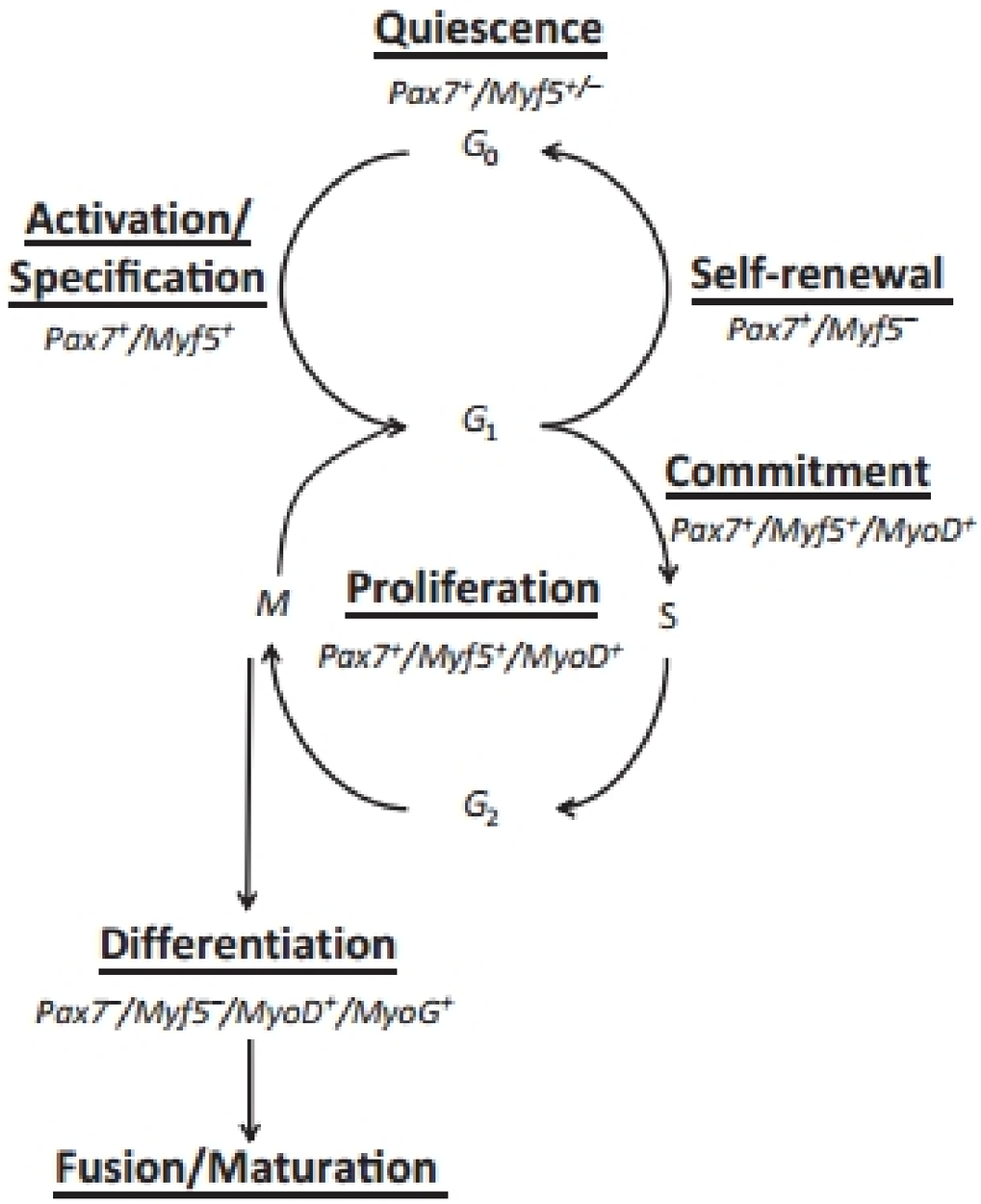


SM SC's: Paracrine & Cytokine Activation Markers

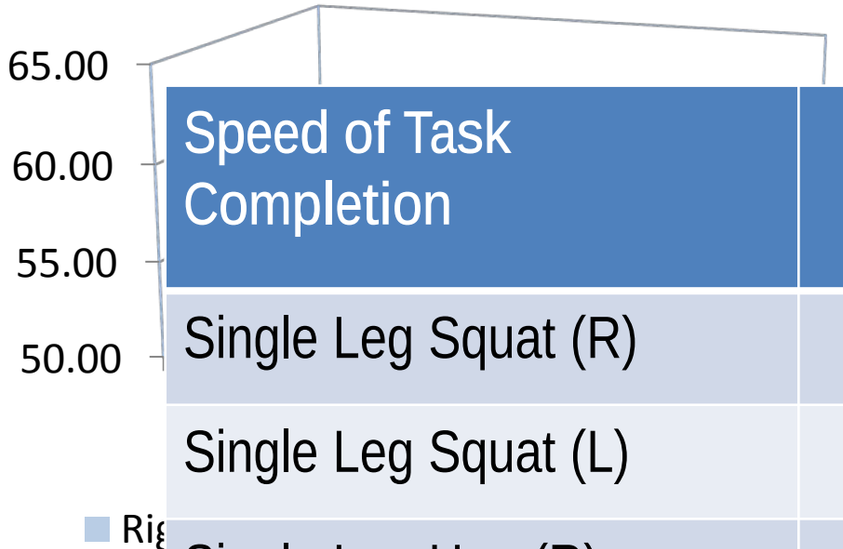


SM: Satellite Cell Activation Cycle



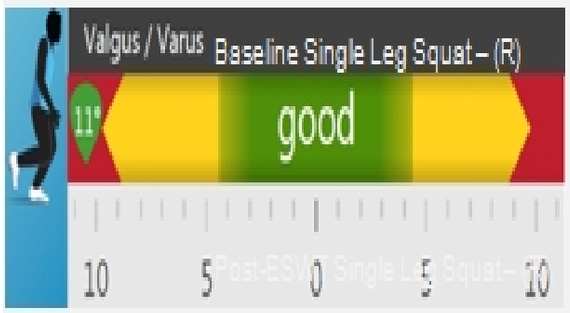


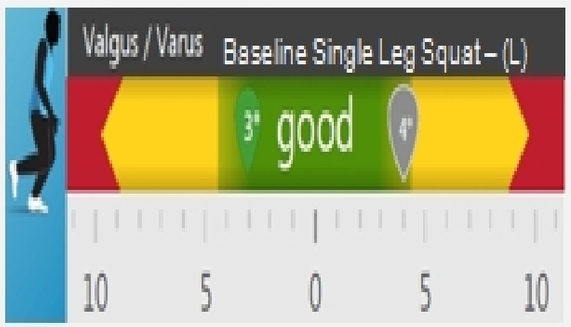
Soccer Medicine Conference: Case Study Result

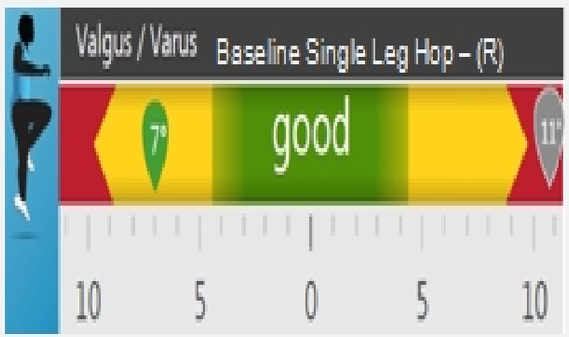


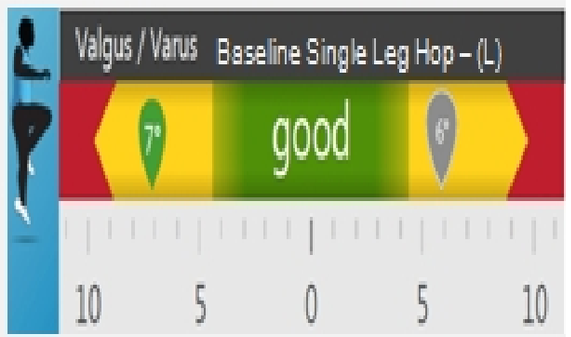
Speed of Task Completion	Baseline	Week 24
Single Leg Squat (R)	UC*	14.02 sec
Single Leg Squat (L)	16.47 sec.	14.03 sec
Single Leg Hop (R)	UC*	23.33 sec
Single Leg Hop (L)	26.09 sec	23.26 sec

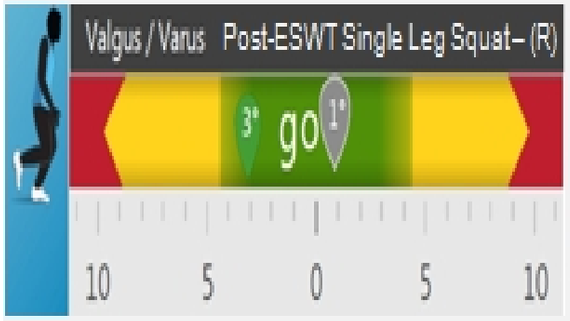
Soccer Medicine Conference: Case Study Result

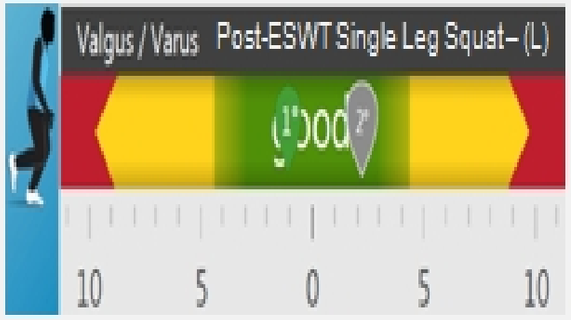


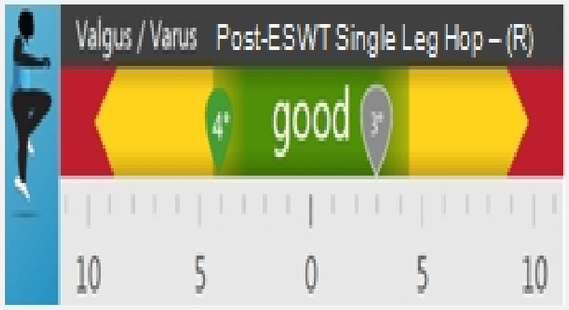


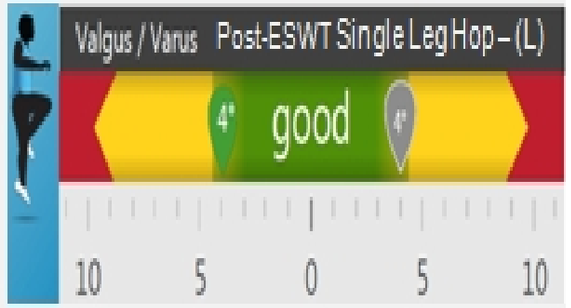












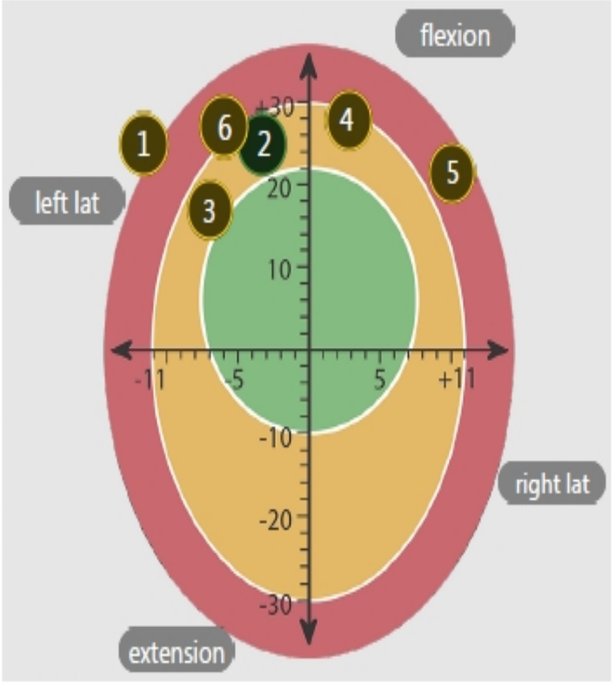
Aging / Sarcopenia Project Pre ESWT

ViMove
Kompass Health Associates

Assessment Date: NOV 11 2015

Functional Live Assessment

Hurdle Step



Score

86

Flexion	
L	R
26°	27°

Lateral	
L	R
-7°	7°

Rep Movement Patterns

Aging / Sarcopenia Project Post ESWT

ViMove
Kompass Health Associates

Assessment Date: Jan 14 2016

Functional Live Assessment

Hurdle Step

Score

53

Flexion	
L	R
7°	8°

Lateral	
L	R
-3°	3°

Rep Movement Patterns

ViMove

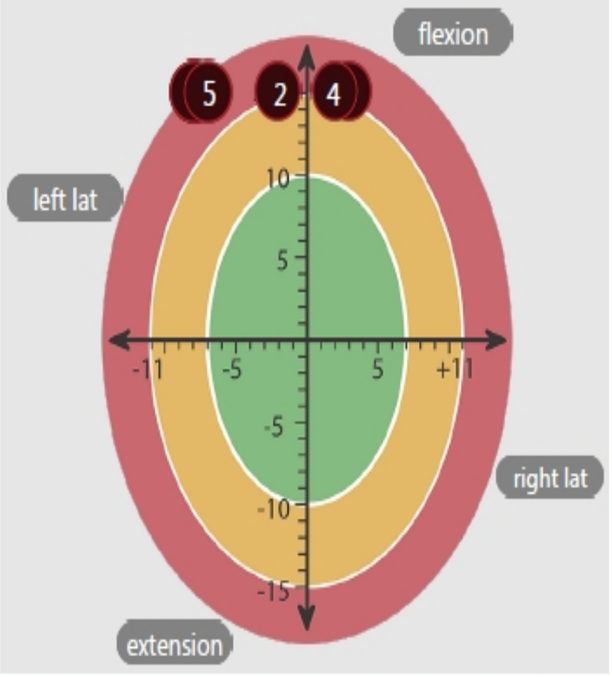
Aging / Sarcopenia Project Pre ESWT

Kompass Health Associates

Assessment Date: Nov 11 2015

Functional Live Assessment

Inline Lunge



Score

82

Flexion	
L	R
27°	29°

Lateral	
L	R
-5°	4°

Rep Movement Patterns

ViMove

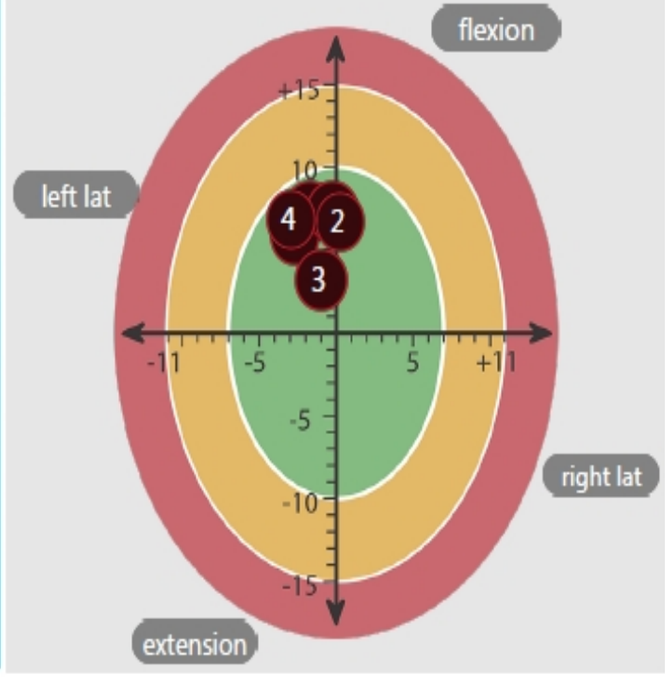
Aging / Sarcopenia Project Post ESWT

Kompass Health Associates

Assessment Date: Jan 14 2016

Functional Live Assessment

Inline Lunge



Score

46

Flexion	
L	R
4°	3°

Lateral	
L	R
2°	1°

Rep Movement Patterns

Test	Baseline	Post Intervention
DorsaVi Move		
Hurdle Step	86	53
Inline Lunge	82	46
Stand from Sit	1.08ec	0.775sec
Weight	424kgs	433kgs
Co-finding (Glycaemic Control)		
Subject #1 67yr Male T2DM	Fast: 141dl / PostPara:195dl	Fast: 133dl / PostPara: 162dl
Subject #2 63yr Male T2DM	Fast: 144dl / Post Para: 198dl	Fast :136dl / PostPara: 170dl
Subject#3 69yr Male T2DM	Fast: 155dl / PostPara: 193dl	Fast:136dl / PostPara: 178dl
*Note: T2DM Subjects on Metformin and insulin comb.		**Lean muscle mass increases approx. 1.8 – 2.2kgs

Summary

Acoustic wave stimulus are seen to:

Summary: Proposed Mechanism of Action

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Review

Shock wave as biological therapeutic tool: From mechanical stimulation to recovery and healing, through mechanotransduction

M.C. d'Agostino [a,*], K. Craig [b], E. Tibalt [a], S. Respizzi [a]

[a] ESWT Center, Rehabilitation Department, Humanitas Research Hospital, Rozzano, Milan, Italy
[b] Kompass Health Associates, Auckland, New Zealand

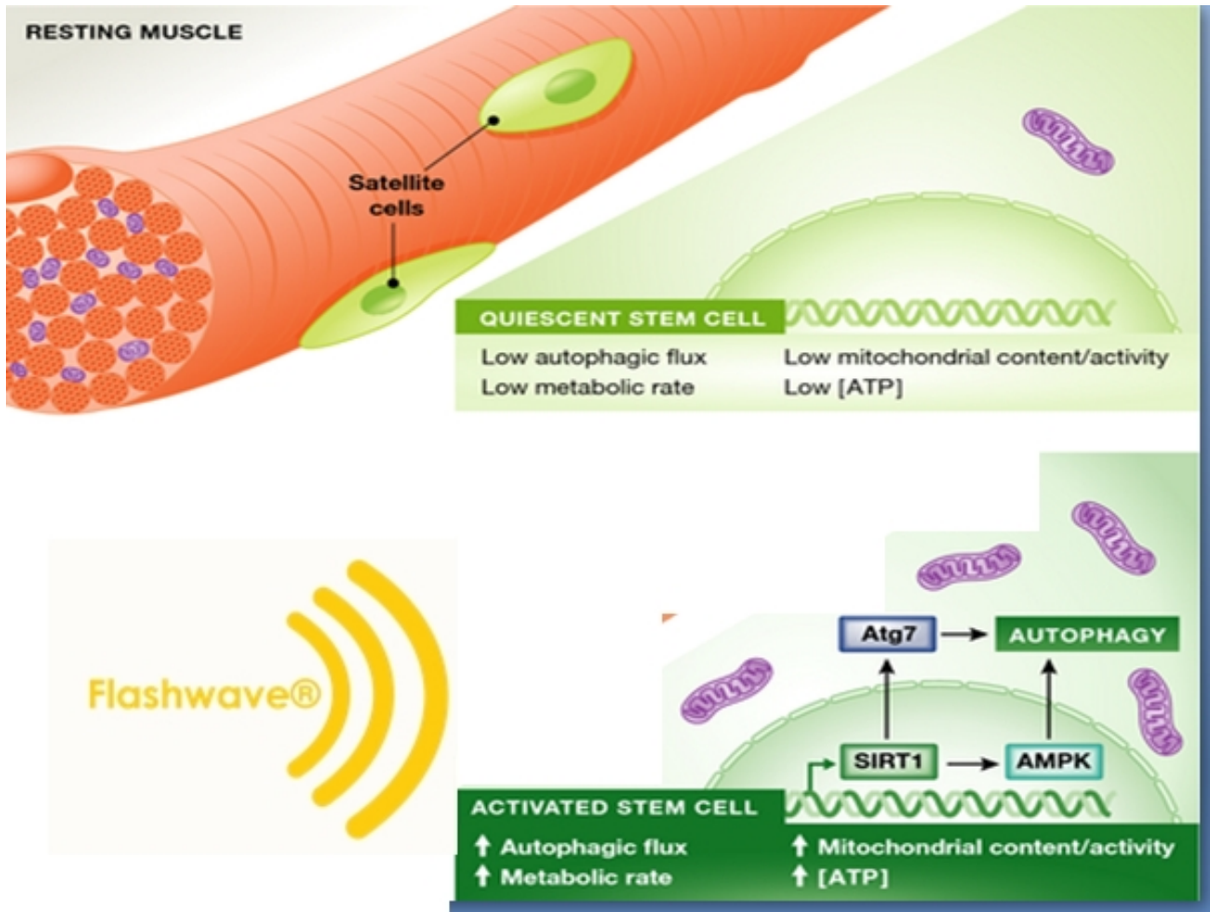
HIGHLIGHTS

- SW represents a revolutionary form of mechanotherapy (acustic stimulation).
- Unlike urological lithotripsy (mechanical model), on living tissues, SW exert an anti-inflammatory action and pro-angiogenic and regenerative effects as well (biological model).
- Mechanotrasduction pathways sustain their clinical and experimental results.
- We present a summary of current knowledge of SW mechanisms of action, according to main recent data (mechanobiology).
- Better comprehension of SW mechanobiology could led to new therapeutical perspectives.

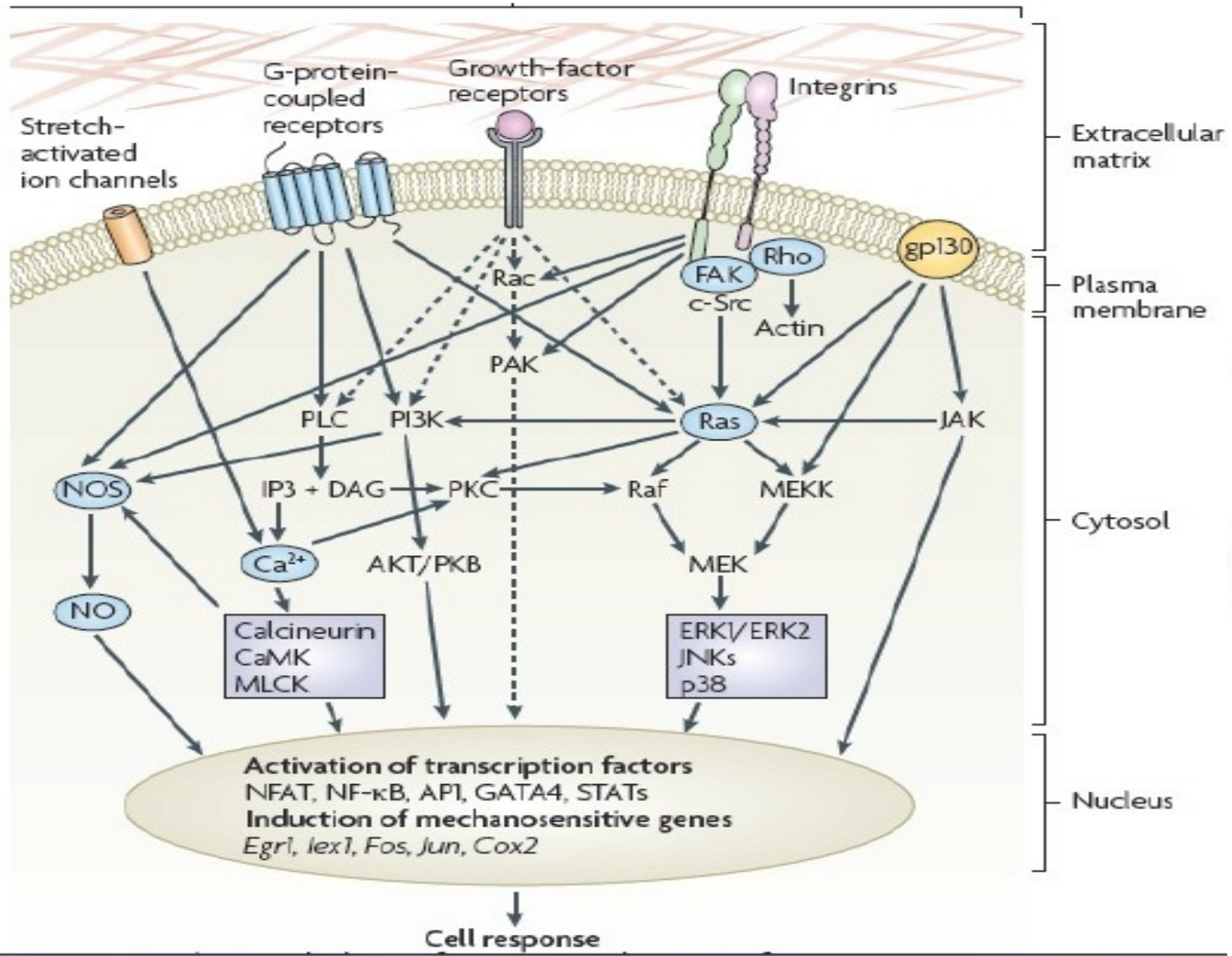
Summary: Proposed Mechanism of Action

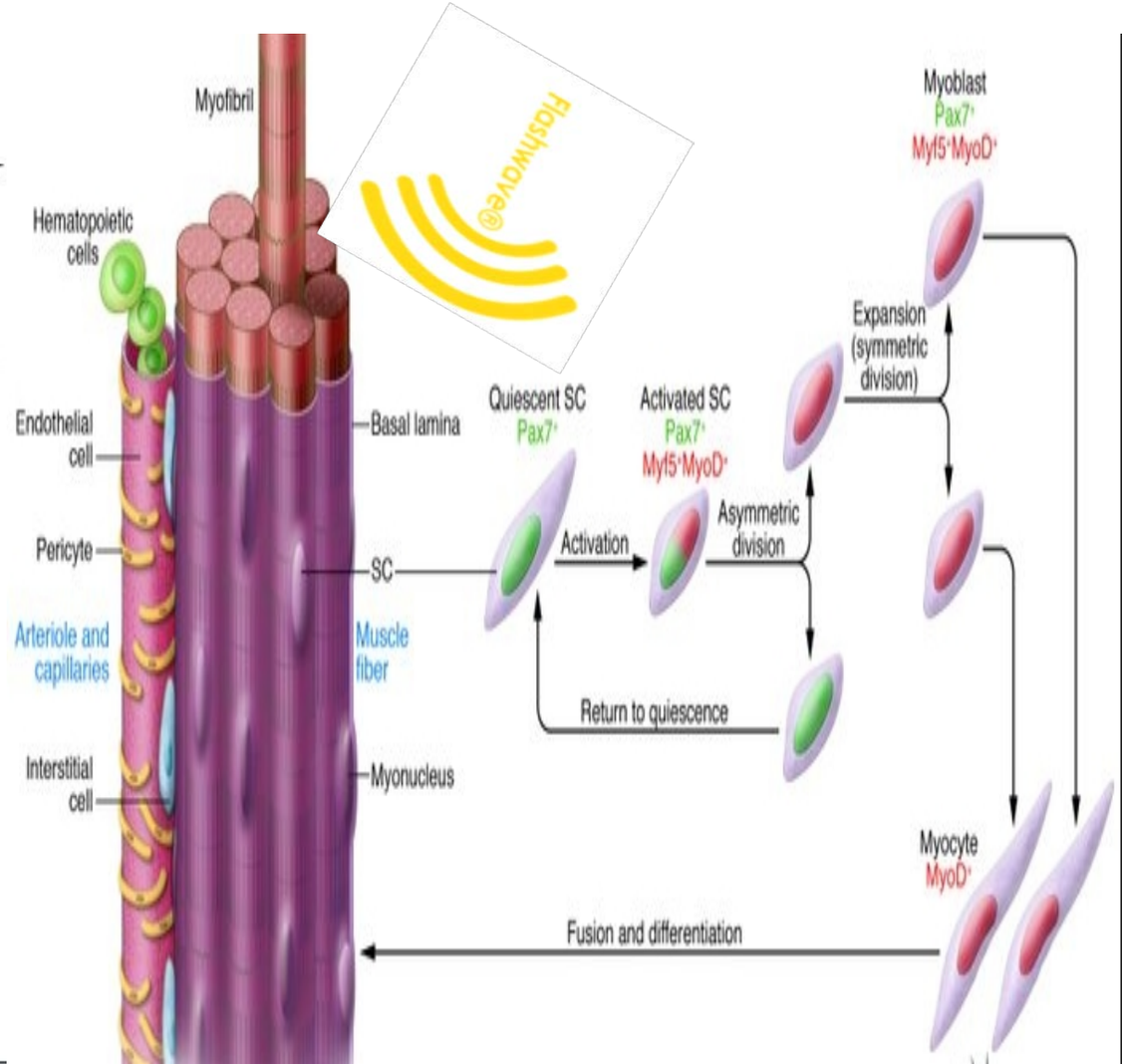
The acoustic stimulus is known to:

- Trigger a biocellular cascade in a process termed ‘bio cellular transduction’
- The cellular sensing occurs between the ECM & intra-cellular matrix; influencing
  - angiogenic factors
  - ion channels
  - integrins / cadherins
  - growth factor receptors
  - myosin motor
  - cytoskeleton filaments
  - improved micro and regional circulation
  - modulation of inflammation
  - regulation of immune factors
  - stem cell activation



Summary: Proposed Mechanism of Action





Summary: Proposed Mechanism of Action

The influence of Flashwave on tissue allows for:

- Tissue regeneration and repair
- Improved tissue resilience
- Functional recovery
- Functional optimisation

Future Applications??

Muscular Dystrophies / myopathies?

- Duchenne’s
- Miyoshi’s
- TIDM Myotonic MD
- Pharyngeal MD
- Metabolic correction in T2DM

Wisdom’s Invitation: *Jer. 33:3*

“Call to Me, and I will answer you,
I will show you great and unsearchable things
that you do not yet know”

Reference

1. Braun T. & Gautel M. Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev Mol Cel Biol. 2011; 12: 349 – 361.

2. Almada EA & Wagers AJ. Molecular circulatory of stem cell fate in skeletal muscle regeneration, ageing and disease. Nat Rev Mol Cel Biol. 2016; 17: 267 – 360..

3. Peault B., Rudnicki M., Cossu G et al. Stem and progenitor cells in skeletal muscle development. Maintenance and therapy. Mol Therp. 2007; 15(5): 867 – 877.

4. Laker RC & Ryall JG. NDA Methylation in Skeletal Muscle Stem Cell Specification Proliferation, and Differentiation. Stem Cell Int. 2016 DOI: 10.1155/2016/5725927

5. Shi X & Garry DJ. Muscle stem cell in development, regeneration, and disease. Genes and Devp. 2006; 20: 1692 – 1707.

6. Chen JCJ & Goldhamer DJ. Skeletal muscle stem cells. Reprod Biol Endocrin. 2003 Open Access Online BioMed Central.

7. Fu X., Wang H & Hu P. Stem cell activation in skeletal muscle regeneration. Cell Mol Life Sci. 2015; 72:1663 – 1677

8. Mitchell KJ., Pannerec A., Cadot B et al. Identification and characterization of a non-satelllite cell muscle resident progenitor during postnatal development. Nat Cel Biol. 2010; 12: 257 – 266.

9. Arecco N., Clarke CJ., Jones FK et al. Elastase levels and activity are increased in dystrophic muscle and impair myoblast cell survival, proliferation, and differentiation. Scientific Reports DOI 10.1038/srep24708

10. Zammit PS., Partridge TA & Yablonka-Reuveni Z. The Skeletal Muscle Satellite Cell: The Stem Cell That Came in From the Cold. J Histochem Cytochem. 2006. 54(11): 1177 – 1191.

11. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr. 2006; 84(3): 475-482.

Reference

12. Armsey TD & Hosey RG. Medical aspects of sports: epidemiology of injuries, preparticipation physical examination, and drugs in sports. Clinics in Sports Medicine. 2004; 23: 255 – 279.

13. Cook JL & Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendiopathy. British Journal of Sports Medicine. 2009; 43: 409 – 416.

14. Yang J, Tibbetts AS, Covassin T, et al. Epidemiology of Overuse and Acute Injuries Among Competitive Collegiate Athletes. Journal of Athletic Training. 2014; 47(2): 198 – 204.

15. Perlick L, Luring C, Bathis C, et al. Efficacy of extracorporeal shock-wave treatment for calcific shoulder tendinitis of shoulder: experimental and clinical results. Journal of Orthopaedic Science. 2003; 8(6): 777 – 783.

16. Lee SY, Cheng B & Grimmer-Somers K. The mid-term effectiveness of extracorporeal shockwave therapy in the management of chronic calcific shoulder tendinitis. Journal of Shoulder and Elbow Surgery. 2011; 20(5): 845 – 854.

17. Vulpiani MC, Trischitta D, Trovato P, et al. Extracorporeal shockwave therapy (ESWT) in Achilles tendinopathy. A long term follow-up observation study. Journal of Sports Medicine and Physical Fitness. 2009; 49(2):171 – 176.

18. Wang L, Lu H-B, Chueng W-H, et al. Extracorporeal shockwave therapy in treatment of delayed bone-tendon healing. American Journal of Sports Medicine. 2008; 36(2): 340 – 347.

19. Notarnicola A & Moretti B. The biological effects of extracorporeal shock wave therapy (eswt) on tendon tissue. Muscles, Ligaments, and Tendons Journal. 2012; 2(1): 33 – 37.

Reference

20. Berta FR. Biological Effects of Extracorporeal Shock Waves on Fibroblast: A Review. *Muscles, Ligaments and Tendons Journal. 2011; 1(4): 137 – 146.*

21. Berta L, Fazzari A, Ficco AM, et al. Effect of extracorporeal shock wave enhance normal fibroblast proliferation in-vitro and activate mRNA expression for TGF-B1 and collegen types I and III. Acta Orthpaedica. 2009; 80(5): 612 – 617.

22. Smutny M & Yap AS. Neighborly relations: cadherins and mechanotransduction. *Journal of Cell Biology. 2010; 189(7): 1075 – 1077.*

23. Huveneers S, Oldenburg J, Spanjaard E, et al. Vinculin associates with endothelial VE-cadherin junction to control force-dependant remodelling. Journal of Cell Biology. 2012;196(5): 641 – 652.

24. Kim J-H, Dooling LJ & Asthagiri AR. Cellular mechanotransduction during multicentre morphodynamics. J R S Interface. 2010; 7(3): S341 – S350.

25. Olivier A, Albiges-Rizo C, Block MR & Fourcard B. Excitable waves at the margin of the contact area between a cell and a substrate. Physical Biology. 2009; 6(20):

26. Sun Y, Chen CS & Fu J. Forcing Stem Cells to Behave: A Biophysical Perspective of the Cellular Microenvironment. Annual Rev. Biophy. 2012; 41: 519 – 542.

27. Geiger B, Spatz JP & Bershadsky AD. Environmental sensing through focal adhesions. Nature Reviews. 2009; 10(1): 21 – 33.

28. Vogel V & Sheetz M. Local force and geometry sensing regulate cell functions. Nature Reviews. 2006; 7(4): 265 – 275.

Reference

29. Raaabe O, Shell K, Goessi A, et al. Effect of extracorporeal shock wave on proliferation and differentiation of equine adipose tissue-derived mesenchymal stem cell in vitro. American Journal of Stem Cells. 2013; 8;2(1): 62 – 73.

30. Leone L, Vetrano M, Ranieri D, et al. Extracorporeal Shock Wave Treatment (ESWT) Improves In Vitro Functional Activities of Ruptured Human Tendon-derived Tenocytes. PloS One. 2012; 7(11): e49759.

31. Smuthy M & Yap AS. Neighbourly relations: cadherins and mechanotransduction. Journal of Cell Biology. 2010; 189(7): 1075 – 1077.

32. Huveneers S, Oldenburg J, Spanjaard E et al. Vinculin associates with endothelial VE-cadherin junctions to control force-dependant remodelling. The Journal of Cell Biology. 2012; 196(5): 641 – 652.

33. D'Angelo F, Tiribuzi R, Armentano H, et al. Mechanotransduction: Tuning Stem Cell Fate. Journal of Functional Biomaterials. 2011; 2: 67 – 87 DOI: 10.3390/jfb2020067.

34. Yim EKF & Sheetz MP. Force-dependant signalling in stem cell differentiation. Stem Cell Research & Therapy. 2012; 3:41 DOI:10.1186/scrt132

35. Holle AW, Tang X, Vijayraghavan D, et al. In situ mechanotransduction via vinculin regulates stem cell differentiation. Stem Cells. 2013; 31(11): 2467 – 2477.

36. Li XH, Deng Z, Ning LN, et al. Roles of Mechanical Force and Mechanotransduction in Fate of Stem Cells. Scientia Sinica Vitae. 2014; 44(7): 639 – 648.

37. DaSilva T.A., De Oliveria Y.A.D., Ferreira J.L.S., Wong R & Lebrao M.L. Sarcopenia According To The European Working Group On Sarcopenia In Older People (WEGSOP) Versus Dynapenia As A Risk Factor For Disability In The Elderly. J Nutr Health Aging. 2014;18(5): 547-553.

38. Pratesi A, Tarantini F & DiBari M. Clin Cases Min Bone Met. 2013;10(1): 11-14.

39. Lizuka K, Machida T & Hirafuli M. Skeletal muscle in an endocrine organ. J Pharmacol Sci. 2014;125(2): 125-131.

Thank you