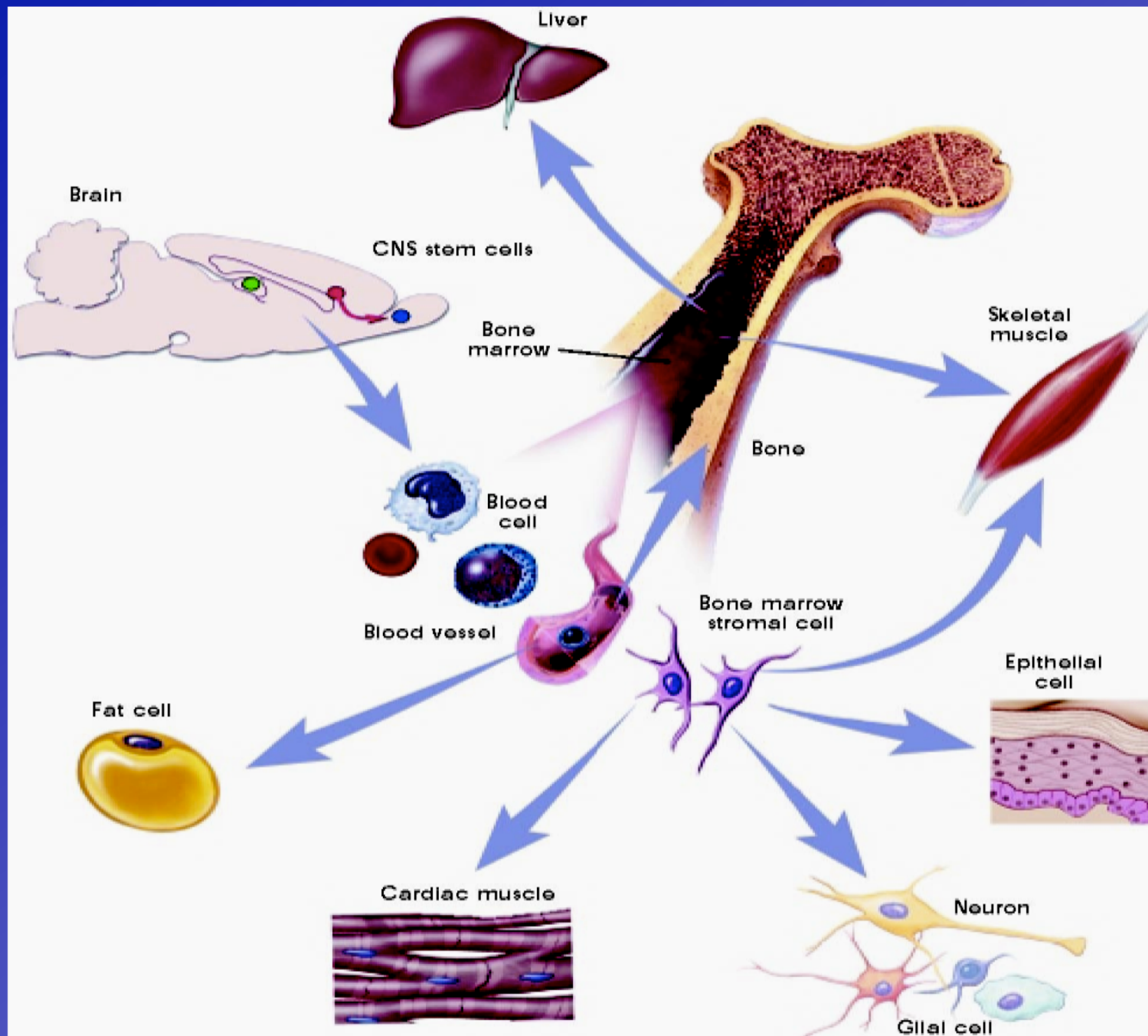
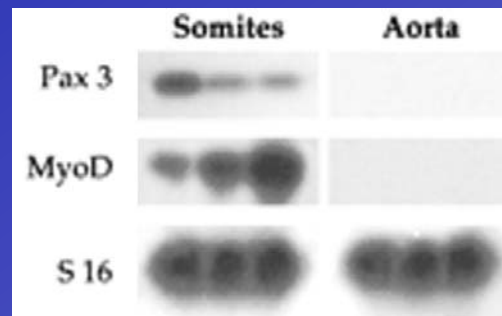
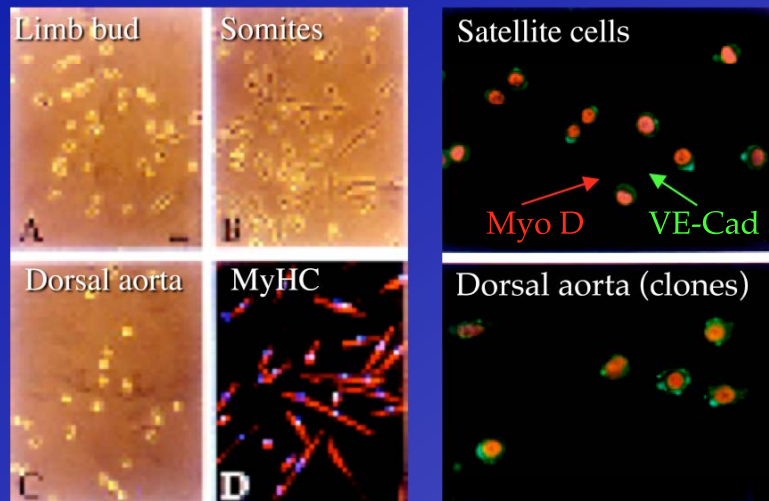


Restoration of microcirculation allows efficient cell therapy at an advanced stage of muscular dystrophy

**Cesare Gargioli
Institute of Cell Biology and
Tissue Engineering
Scientific Park of S. Raffaele
Rome**



Mesoangioblast from embryonic dorsal aorta



Cell type: Aorta Satellite Myoblast

MyoD	+	+	+	
Myf5	+	+	+	
Desmin	+	+	+	
MNF	+	+	+	
C-Met	+	+	+	
VE-Cad	+	+	-	
Flk-1	+	+	+	
P-selectin		+	+	-
PECAM		+	+	-
CD34	+	+	-	
SMA	+	+	+	

Endothelial

(Flk+, PECAM+)

Pericytes

(Flk-, PECAM-)

Vessel associated progenitors clonally expanded

1. Dissect dorsal aorta from E9.5 mouse embryo



2. Grow the aorta as organ culture for 7-15 days



3. Dissociate to single cells and clone on STO feeder layer

4. Pick clones with given morphology and expand

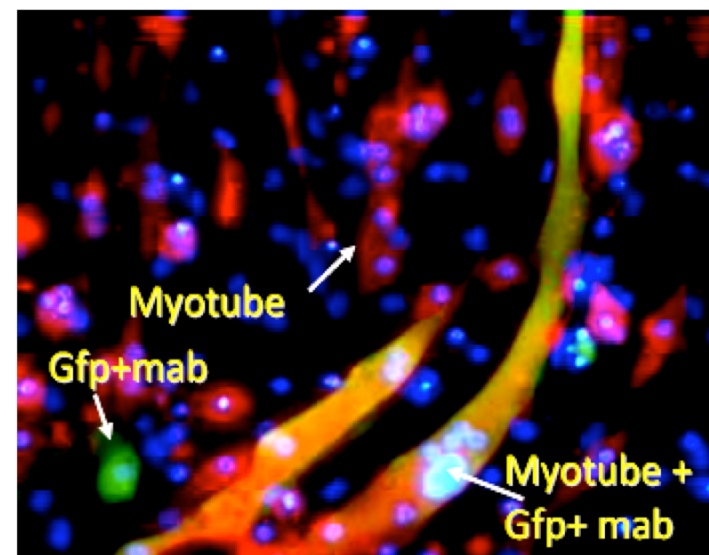
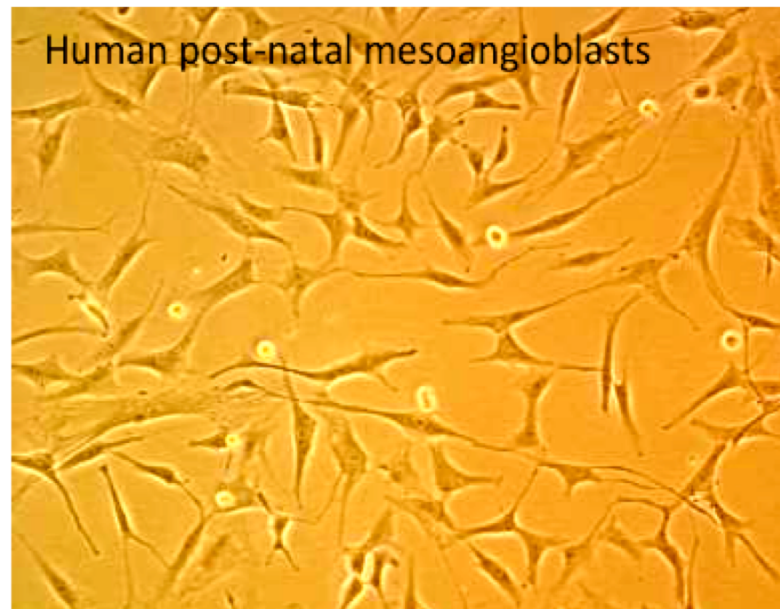


Mesoangioblasts

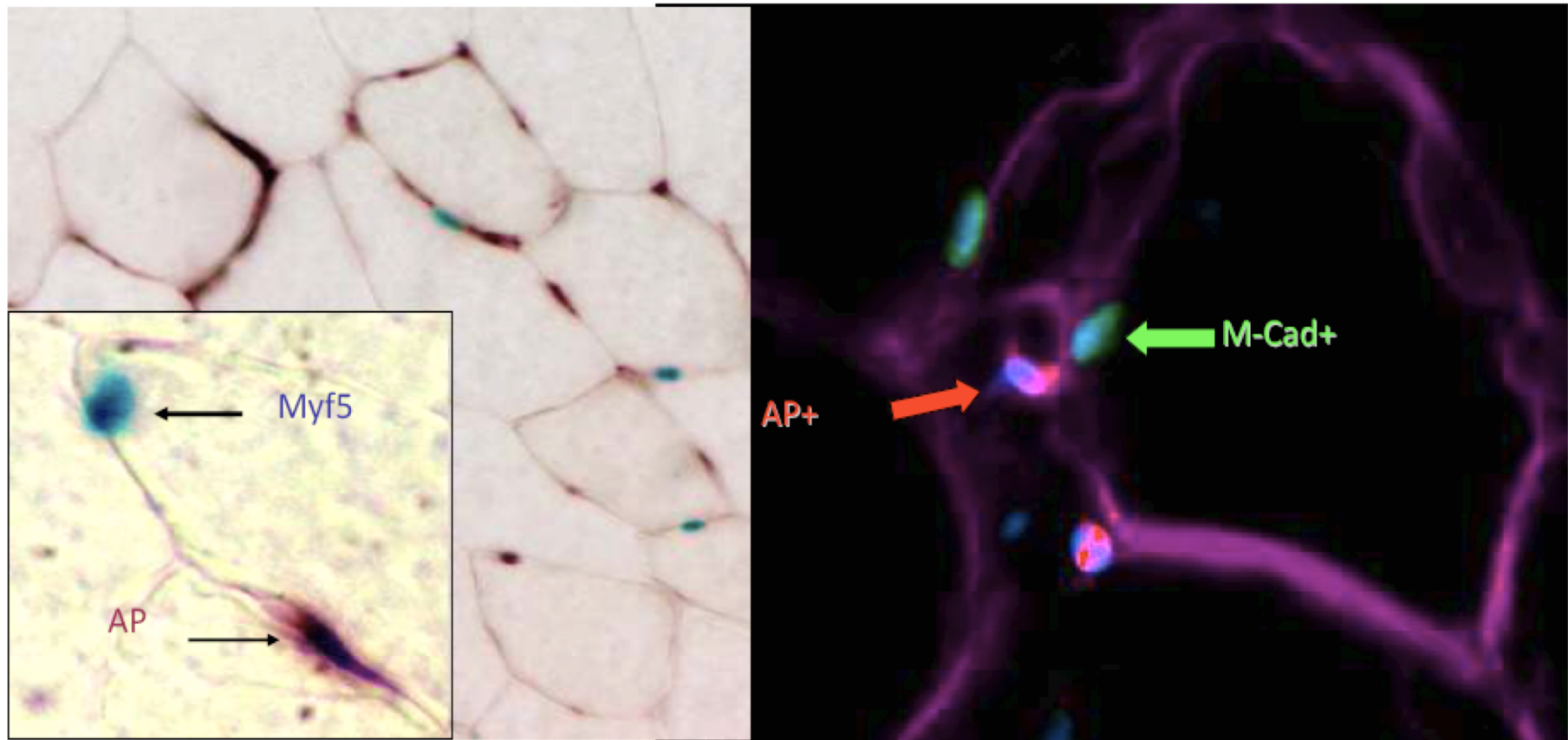
Can be isolated from Mouse, Dog and Human embryonic and post-natal vessels, cloned and expanded in vitro for approximately 20 PD (except than in rodents)

Express endothelial markers (CD34, Sca1, VE-Cad, Flk-1 and c-Kit) when isolated from the embryo; however they express pericyte markers (NG2, AP, PDGF-Rb, SMA) when isolated from post-natal tissues.

Differentiate into smooth, cardiac or skeletal muscle



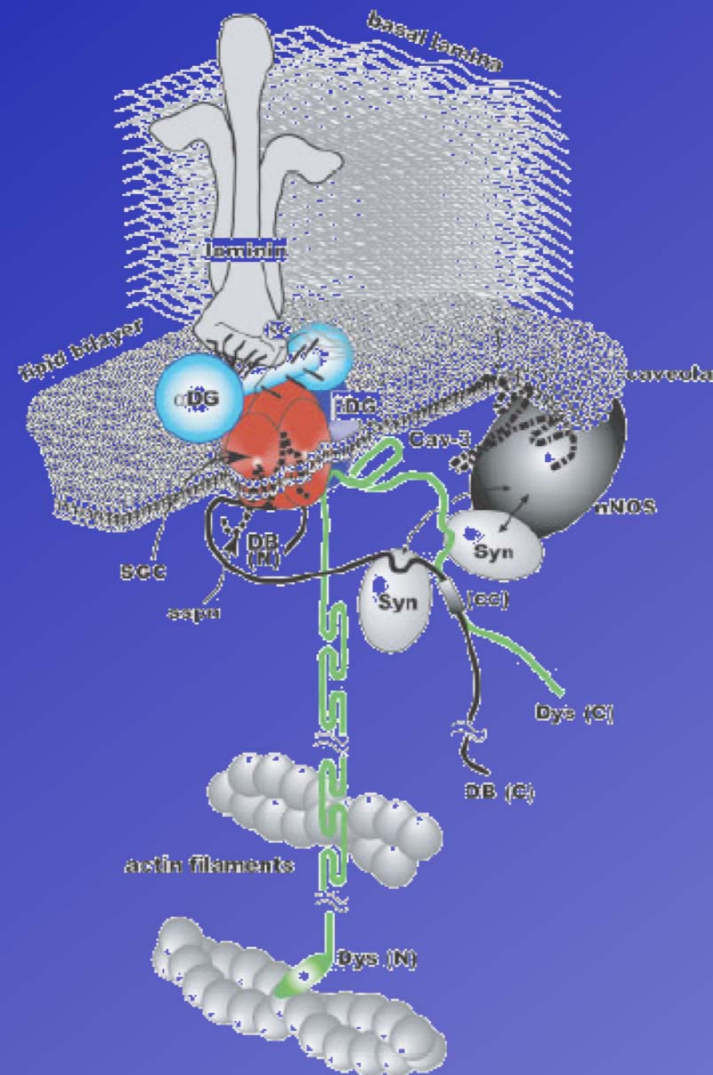
Human and mouse post-natal pericytes express Alkaline Phosphatase (AP) in vivo



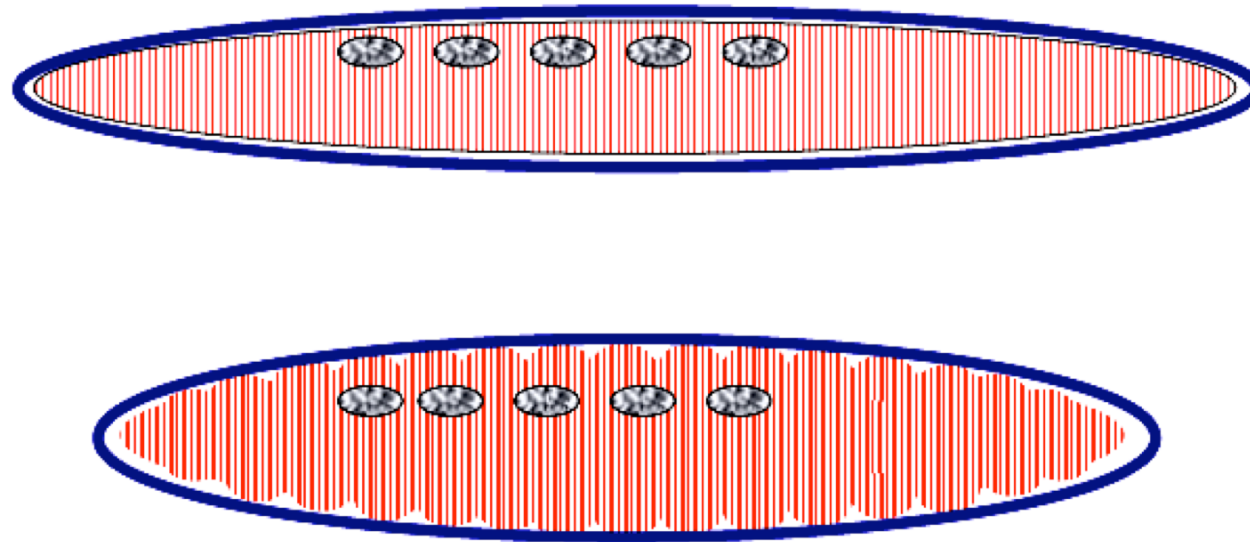
Gene expression of mesoangioblast clones

	A1	A2	A4	A6	B13	D16
CD34	+	+	+	+	+	+
c-kit	-	-	-	-	-	-
Sca-1	+	+	+	+	+	+
Flk-1	-	-	-	-	-	-
Flt-1	-	-	-	-	-	-
Tie-2	+	+	+	+	-	-
Myf-5	-	-	-	-	-	-
Myo-D	-	-	-	-	-	-
Pax-3	+	+	+	+	+	+
Pax-7	-	-	-	-	-	-
Emx2	+	+	+	+	+	+
VE-Cad	+	-	+	+	+	+
Mef-2D	+	+	+	+	+	+
Gata-4	+	+	+	+	+	+
Nkx-2.5	-	-	-	-	-	-
Oct-4	-	-	-	-	-	-

Dystrophy recovery (α SG-KO mice model)

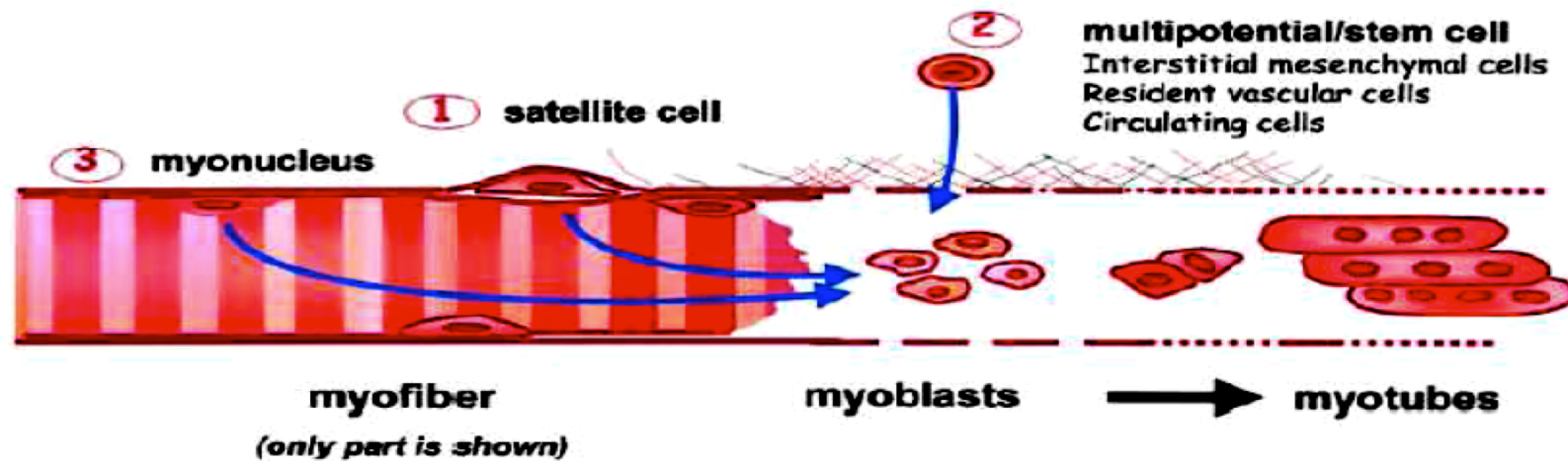


During contraction, the muscle fiber membrane shortens while remaining connected to both cytoskeleton and basal lamina

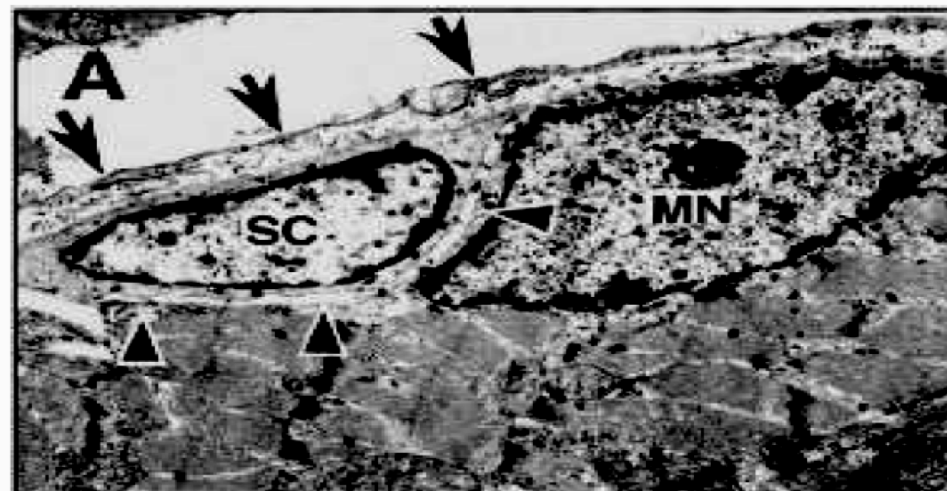


In the absence of the dystrophin complex the membrane has increased chances of being damaged during contraction

Damaged or dead fibers are repaired or replaced by satellite cells and, to a lesser extent, other cells



Satellite cells were originally identified for their position underneath the basal lamina but outside the plasma membrane of the muscle fiber



Duchenne Muscular Dystrophy

➤ X-linked recessive disease caused by mutation of dystrophin gene, the largest known gene (2.4 Mb) coding for a 427 kD cytoskeletal protein

➤ It is the second most common genetic disorder in man and the most common muscular dystrophy of childhood (incidence 1:3500 male live births)

➤ Characterized by degeneration of skeletal muscle due to absence of dystrophin on muscle fibre membrane

