

Beyond Transformation: Skeletal Muscle Satellite Cell as a Possible Source of Neomyocardium

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Abstract

For the past decade, skeletal muscle has been transformed into predominantly type I, highly fatigue resistant, aerobic muscle fibres to be used for cardiac assist, either in the form of dynamic cardiomyoplasty or skeletal muscle ventricle [11, 13]. In spite of such transformation, the muscle retains many of the structural, functional and electrophysiological characteristics of a skeletal muscle [11-43]. More fundamental alteration of skeletal muscle for cardiac assist may be possible if we focus on its stem cells rather than the mature, already differentiated skeletal muscle fibres. Our lab has been interested, for the past few years, in the study of muscle regeneration with the specific application of using satellite cells as a means of myocardial repair. In this article, we will review the development of our understanding of skeletal and cardiac regeneration, satellite cell anatomy and biology, and address the issue of terminal differentiation of the muscle cell. Based on this knowledge, we will discuss the feasibility of transplanting satellite cells into injured myocardium with the intent of repair.

Key words: Satellite cell transplantation, Neomyocardium, Cardiac muscle repair, Cardiac muscle substitution, Heart.

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Historical overview

Descriptions of skeletal muscle regeneration date back to the turn of the century [2, 10]. Early observations concerned themselves with trying to identify the process of regeneration itself and the sequence of events involved in muscle repair [79]. In 1934, Millar described complete skeletal muscle regeneration following injury using an animal model [50]. In the description of the events he observed, he identified proliferating nuclei but he could not clearly determine the source of the regenerate. Twelve years later, Le Gros Clark described a model of regeneration in which pieces of skeletal muscle were resected and then grafted *in situ* [40]. He observed the presence of signs of regeneration as early as one week following implantation, and the presence of young fibres undergoing maturation 2-3 weeks later. He specified that the regenerated fibres keep their orientation with respect to the dying fibres suggesting a directive action of endomysial tubes surviving injury. This has been confirmed in recent studies [4, 5, 77]. Finally, he suspected that myoblasts were derived from disintegrated fragments of muscle but was not able to demonstrate this. Gay and Hunt, in 1954 [11], described

the maturation of regenerating skeletal muscle fibres as observed in phase microscopy. They compared regeneration to ontogenic development and described centrally placed nuclei moving to the periphery of fibres as they mature. They alluded to single cells fusing to form a new fibre.

In 1961, Mauro described the skeletal muscle "satellite" cell and suggested that these cells may be the source of nuclei for muscle regeneration. This process mimics embryonic muscle development in which individual mononucleated undifferentiated, uncommitted myoblasts undergo multiple cell divisions and then fuse to form muscle fibres in which the post-mitotic nuclei are exclusively concerned with protein synthesis [10, 34, 41, 51, 74, 76]. Growth then depends on further replication of the satellite cells which persist into adult life [53]. In muscle regeneration, fibres first undergo degeneration. The post-mitotic nuclei, responsible for metabolic activity, do not contribute to the regenerative response [61]. In contrast, satellite cells survive injury and become myogenic [75, 77]. The newly formed muscle then undergoes a maturation process [77].

Biochemically, the lactate dehydrogenase and creatine kinase isozyme profiles share similarities but are not exact duplicates of the ones observed in embryonic differentiation [77, 83]. This has been interpreted as being due to the harsher metabolic environment of regenerating muscle in comparison to developing muscle.

Cardiac muscle

Cardiac muscle resembles skeletal muscle in that it is striated [76, 82]. In contrast, the cells are mononucleated and they form a syncytium in which they are connected by specialized cell junctions referred to as intercalated discs. Its metabolism differs from that of skeletal muscle in that it is fatigue resistant [9]. Thus, myocardium is more differentiated than skeletal muscle and is highly adapted to function. Embryogenesis of heart muscle occurs earlier than that of skeletal muscle. Initially, myocardium consists of developing muscle cells that originate from splanchnic mesoderm. As cardiac myoblasts mature, they synthesize myofibrils which align themselves progressively [44]. The cells then elongate and intercalated discs form a right angle with respect to the fibrils. Cardiac myoblasts are able to both synthesize myofibrillar proteins and divide [88]. This is in contrast to skeletal muscle in which muscle specific gene expression precludes mitotic cycling [3, 59]. Lastly, mitotic activity is lost in the neonatal period, and following birth, the heart enlarges only by hypertrophy of its myocytes.

Regeneration of myocardium following injury is poorly defined [8, 14, 20, 36, 48, 56, 73, 88]. Cytokinesis has not yet been described. Following cell death, myocardial repair essentially consists of replacement with connective tissue and inevitably, loss of function ensues. This inability for repair or "regeneration" following injury may be circumstantially attributed to the absence of satellite cells in mammalian cardiac muscle [7]. Such cells have been observed in cardiac muscles of decapod crustaceans which consist of multinucleated fibres joined together by intercalated discs [49].

Several studies have attempted to characterize the potential for regeneration in myocardium. Long term culture of adult mammalian cardiac muscle cells have revealed that these cells undergo biosynthetic and morphological changes to eventually resemble embryonic cardiac cells [15, 22, 57]. They resume DNA synthesis and about 90% of these cells contain more than one nucleus [58]. Looking at DNA synthesis studies in response to experimental injury carried out on neonatal rats, Nag et al. showed an age dependent potential for regeneration that becomes attenuated by about 4 weeks of age [56]. In this study, the regenerating cardiac myocyte was characterized as a cell with many polysomes presumably synthesizing new myofibrils. Still, mitotic division was not observed. Oron and Mandelberg reported similar intracellular regeneration following cryoinjury. Their findings were limited to the perinecrotic area [60]. Such forms of regeneration have been confirmed in human autopsy studies [47]. Others have tried to focus on the transplantation of cardiac tissue

into ectopic sites. Jockusch et al. showed that xenografts of ventricular tissue from newborn rats implanted into an athymic host were able to regenerate [29]. Thus, the common denominator linking these studies is that although newborn heart muscle may have some capacity for limited regeneration, mature heart muscle cannot repair itself following injury.

The satellite cell

As alluded to, hope for myocardial regeneration may lie in our understanding of the satellite cell. Originally described in frog muscles [46], these cells are found in all known mammalian voluntary muscle. Their creatine kinase (CK) enzyme profile resembles that of embryonic myoblasts [5]. They are absent in cardiac and smooth muscle [46]. Physiologically, they have the ability to divide and give rise to differentiating cells as well as new satellite cells. Their nuclei constitute the only ones among striated muscle nuclei that have the capacity for mitosis [5, 40, 48, 49, 53, 68]. As well, they have the ability to cycle *in vitro* [1]. Thus, these cells can be defined as stem cells of embryonic origin [48].

Using electron microscopy, satellite cells are distinguishable by their position beneath the basal lamina [53]. The plasma membrane of these cells is separated from that of the "parent" fibre by a gap 15-20 μ wide. The cell nucleus itself is slightly smaller than a myofibre nucleus. In general, human satellite cells measure 30 μ in length [37]. They make up about 5% of nuclei in muscle fibres. This has been confirmed in laboratory animals as well as in humans [64, 65].

In mature muscle, satellite cells appear to be clustered near the motor end-plate [33]. They are not, however, evenly distributed among the fibre types and trends have not been identified according to muscle fibre type composition [29]. Such proportional differences between various muscle groups appear to be related to nerve supply [66].

The morphology of satellite cells was described in the mid 1960's [54]. In mature muscles, few organelles are seen. The width of the cell itself is slightly greater than that of the nucleus and the shape is fusiform. Occasional Golgi apparatus can be found near the nucleus and small, poorly developed mitochondria can be seen at the tapering ends of the cell. Free ribosomes can be found within the limited cytoplasm. Finally, centrioles are present at the side of the nucleus, thus reflecting the ability that these cells have for mitotic cycling.

In growing muscle, satellite cells appear to be more metabolically active [67]. The cells lie beneath the basal lamina of muscle fibres but are more conspicuous in that the plasma membranes of the satellite cells and the muscle fibres are not parallel to each other and are separated by a gap up to 60 nm wide. Ribosomes are numerous and are both free and bound to endoplasmic reticulum. The Golgi apparatus is well developed. Finally, some satellite cells can be observed to be in the process of fusion with a muscle fibre. Eventually, with age, the cells evolve to take on a morphology that reflects their quiescent nature.

Laboratory animal and human studies have confirmed that adult satellite cells revert to their active form after being stimulated by stresses such as disease, direct trauma, ischemia or exercise [7, 64, 65]. Regenerating skeletal muscle is solely dependent upon the intrinsic satellite cell population that survives injury [4, 35, 70]. Furthermore, the myogenic potential of satellite cells decreases with age and multiplicity of regenerating cycles [62, 72]. Within the basal lamina, satellite cells have the ability to migrate toward the injury site [71]. This suggests the possible role of muscle injury in releasing factors that can activate satellite cells and guide them to the injury site [6].

One question that arises in discussing satellite cells is whether or not these cells are identical to embryonic myoblasts. Much of the evidence available suggests that although satellite cells resemble embryonic muscle cells, they are most likely a subpopulation of these [12, 16, 17, 18, 45, 84]. As stated above, adult satellite cells have the same levels and CK isozyme profiles as embryonic myoblasts. The predominant form is BB and, as in embryonic muscle, the MM isozyme accounts for the increase in CK activity observed during fusion leading to formation of multinucleated fibres. Analysis of muscle regeneration has shown that muscle satellite cells synthesize embryonic isoforms of myosin and tropomyosin. This suggests that in muscle regeneration originating from satellite cells, the differentiation sequence is similar to that of embryonic muscle [12]. *In vitro*, satellite cell derived myotubes from fast muscle synthesize only fast myosin light chains, while myotubes derived from slow muscle satellite cells synthesize both fast and slow light chains [45]. This indicates that satellite cells are to some extent more differentiated than primitive embryonic myoblasts. Such a concept of heterogeneity among myogenic cells is consistent with the observation that acetylcholine receptors are expressed at all stages by satellite cells but appear in embryonic cells only at the onset of terminal differentiation [16, 84]. Moreover, 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter, can be used to suppress myotube formation in embryonic myoblasts. When the same treatment is applied to cultures of adult satellite cells or myoblasts from older embryos, it doesn't suppress myotube formation. Thus, embryonic muscle histogenesis appears to be a sequence of distinct phases that are reflected in the consecutive appearance of various subpopulations of myogenic cells, one of which is represented by satellite cells.

Satellite and embryonic muscle cells can be further distinguished from each other by their morphology upon withdrawal from mitotic cycling [18]. In culture, the transition from the proliferative stage to the post-mitotic state is associated with a change from a round shape to a spindle shape in satellite cells. Embryonic myoblasts, on the other hand, always appear spindle shaped. Interestingly, using tritiated thymidine labelling and radio-autography, it was found that myoblasts and satellite cells were able to recognize each other and fuse to form hybrid myotubes when co-cultured together. Finally, at the onset of myogenesis

in regenerating muscle, satellite cells divide as little as once before fusing into multinucleated myofibres [24, 25, 62]. This is in contrast to embryonic myoblasts which divide at least four times before terminally differentiating.

Terminal differentiation

We hypothesize that terminal differentiation of muscle cells can be influenced. Myogenic differentiation can be defined as the process by which myoblasts become post-mitotic and subsequently fuse to form myotubes which express muscle specific genes. Growth factors are able to interact with specific receptors on the cell surface and by this mechanism induce intra-cellular signalling which can affect both proliferation rate and differentiation. These effects can be induced in many cell types and various combinations of growth factors may have additive effects. Although discussion of specific growth factors is beyond the scope of this article, a recent paper by Egballi et al. is representative of this growing field of study [21]. These authors describe a series of experiments in which cultured cardiac fibroblasts treated with transforming growth factor B, can display a myocyte phenotype and express sarcomeric actin mRNA. One can only speculate on the implications of such an observation, but this may mean that future studies could identify new regulatory factors for muscle cells that may be different from those that are already known [19, 63, 78, 86, 87]. Such regulatory factors are described as having distinct biological roles in controlling muscle development.

The final stage of maturation depends on innervation of the developing myotubes. This has also been confirmed for skeletal muscle regeneration [27, 55]. Experiments have shown that fast twitch muscles could be converted to slow twitch muscle by cross-innervation with a slow twitch nerve [31]. Such a transformation is accompanied by changes in regulatory proteins of the muscle cells, isozyme profiles, and histochemical characteristics. Thus, alteration of innervation leads to alterations in the translation or transcription activities of the cell. Ongoing studies in our laboratory have involved the transformation of mature skeletal muscle using electrical stimulation. This results in conversion of the contractile and calcium regulating systems to profiles approaching those of cardiac muscles [28].

Studies from the 1960's and early 1970's established that skeletal muscle fibres are formed via fusion of mononucleated myoblasts [34, 53]. In 1965, Yaffe and Feldman examined the possibility of fusion of cells of different genotypes using radio-autographic cell labelling techniques [85]. They were able to show *in vitro* production of hybrid muscle fibres using myoblasts from calf fetuses and newborn rabbits and rats. These findings suggest the possibility of using cell transplantation in a way that may alter genetic make-up of muscles.

In 1979, Jones as well as Lipton and Schultz developed experimental models which combined *in vitro* and *in vivo* methods [30, 40]. Isolated myogenic cells from regenerating adult rat skeletal muscle were grown in culture, labelled with tritiated thymidine and grafted back into adult

skeletal muscle. Using such a model, Jones was able to show limited survival of homografts (implantation into a different host of the same species) and long term survival of autografts. This model suggested that cells rather than tissue could be transplanted for organ repair and that immune response might be expected with such manipulations. Indeed, Karpati et al. have shown that major histocompatibility complex gene products are expressed in regenerating human skeletal muscles [32]. In the last twelve years, others have confirmed that implanted satellite cells multiplied *in vitro* retain their myogenic potential, participate in regeneration of injured skeletal muscle and could co-express at least some of their genes with host nuclei within the same muscle fibre [1, 26, 52, 80, 81]. Law et al. have shown that myoblasts from embryos could be used to improve structure and function of dystrophic muscles [37, 38, 39]. Furthermore, satellite cells multiplied *in vitro* have been used to regenerate skeletal muscle following experimental injury [1, 70]. Clinically, although the results are not known, trials have been undertaken in humans to use satellite cell transplantation in the treatment of muscular dystrophy (Karpati, G.: Montreal Neurological Institute - Personal Communication).

Myocardial repair

From our review of the literature, it is clear that satellite cells are the basic unit of muscle regeneration. It is also clear that these cells can be transplanted successfully. There is also evidence that terminal differentiation of muscle cells can be influenced depending on the neuroendocrine environment of the cells. Based on this knowledge, we have autotransplanted satellite cells into injured myocardium. Our early results suggest regenerating cardiac muscle fibres are present. This finding needs further confirmation using appropriate cell markers. In one particular implant specimen, the muscle fibres found within the injured myocardium look very similar to skeletal muscle, with the absence of intercalated discs and peripherally located spindle shaped nuclei. Dispersed within the fibres, however, are nuclei characteristic of cardiac muscle cell. We interpret this as a skeletal-cardiac mosaic muscle fibre. This reflects the possibility that the satellite cells were implanted just prior to the point of myotubule formation. We suspect that some of these cells were near the point of terminal differentiation along the skeletal muscle line while others were not, allowing them to develop morphologic features characteristic of cardiac myocytes. The mosaic arose because of the influence of the intracardiac environment on these near-differentiated cells.

We conclude that the application of satellite cell implant, both in skeletal muscle and cardiac muscle, is promising and warrants further investigation.

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Skeletal muscle as a source of neomyocardium

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