

# The role of satellite cells in skeletal muscle regeneration

## Rola komórek satelitarnych w regeneracji mięśnia szkieletowego

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### Key words

satellite cells, myoblasts, myogenic regulatory factors, self-renewal, cell transplantation

### Abstrakt

Skeletal muscles are composed of multinucleated fibers that cannot divide. They retain the ability to regenerate due to the presence of mononucleated cells, called satellite cells. Mitotically quiescent satellite cells are located between the sarcolemma and the basal lamina of the muscle fiber. They can be activated in response to muscle injury. Then they proliferate, differentiate and eventually fuse to damaged myofibers or fuse together to form new myofibers. A part of the activated cells escape differentiation and restore the pool of quiescent satellite cell under the basal lamina of the newly formed muscle fibers. A decline in the number and activity of satellite cells results in an impaired regeneration potential of aged muscle. However regeneration can be improved by modification of the microenvironment of the skeletal muscle. Satellite cells have been considered as a source for cell-based therapies in the treatment of diseases such as muscular dystrophies, heart failure, insufficient function of external urethral sphincter. They can be derived easily from skeletal muscle biopsies and cultured in vitro before cell transplantation. After injection to the host muscle they undergo a myogenic differentiation program. In the case of muscle dystrophy, clinical trials have demonstrated a lack of health improvement after transplantation. This was the result of the poor survival and limited migratory capacity of the injected cells. More promising results were obtained when satellite cells were transferred to the locally damaged muscles. This article demonstrates the role of satellite cells in skeletal muscle regeneration, and the possibilities of their use in cell-based therapies in the case of diseases where muscle fibers are impaired.

### Słowa kluczowe

komórki satelitarne, mioblasty, mięśniowe czynniki transkrypcyjne, samoodnowa, przeszczepy komórkowe

### Streszczenie

Mięśnie szkieletowe składają się z wielojądrowych włókien, które nie podlegają podziałom komórkowym. Zachowują jednak zdolność do regeneracji dzięki obecności populacji jednojądrowych komórek zwanych komórkami satelitarnymi. Nieaktywne mitotycznie komórki satelitarne zlokalizowane są pomiędzy sarkolemmą a błoną podstawną włókna mięśniowego. Są one aktywowane w wyniku uszkodzenia mięśnia. Wówczas ulegają procesom proliferacji, różnicowania i ostatecznie łączą się ze zniszczonymi włóknami mięśniowymi lub też łączą się wzajemnie ze sobą formując nowe włókna. Część aktywowanych komórek wycofuje się z procesów różnicowania, powraca do stanu nieaktywnego i zostaje zdeponowana pod błoną podstawną nowo uformowanego włókna odnawiając w ten sposób pulę komórek satelitarnych. Spadek liczby oraz aktywności komórek satelitarnych odpowiedzialne są za osłabioną wydajność regeneracji starzejących się mięśni. Jednakże proces regeneracji może zostać usprawniony poprzez modyfikacje mikrośrodowiska mięśnia szkieletowego. Komórki satelitarne postrzegane są jako materiał do terapii komórkowych w przypadku takich chorób jak dystrofia mięśniowa, niewydolność mięśnia sercowego, niedomoga zewnętrznego zwieracza cewki moczowej. Mogą być łatwo pozyskane z biopsji mięśnia szkieletowego a następnie hodowane in vitro zanim zostaną poddane transplantacji. Po przeszczepie komórki satelitarne rozwijają się zgodnie ze schematem typowym dla komórek mięśnia szkieletowego. W przypadku dystrofii mięśniowych próby kliniczne nie wykazały poprawy zdrowia pacjentów po transplantacji komórek. Spowodowane jest to słabą przeżywalnością komórek po przeszczepie oraz ich ograniczoną dystrybucją poza miejsce wstrzyknięcia. Bardziej obiecujące wyniki uzyskano w przypadku wykorzystania komórek satelitarnych dla przywrócenia sprawności mięśniom uszkodzonym miejscowo. Celem niniejszej pracy jest ocena udziału komórek satelitarnych w regeneracji mięśnia szkieletowego oraz ich wykorzystania w terapiach komórkowych chorób, związanych z upośledzeniem czynności włókien mięśniowych.

The letters indicate the authors' contribution to the paper: A – research project; B – data collection; C – statistical analysis; D – data interpretation; E – work on manuscript; F – literature search; G – funds procurement

Article received 25.03.2009; accepted for publication: 12.10.2009

## Introduction

Skeletal muscles constitute 40-45 % of the body mass of an adult person<sup>1</sup>. They are responsible for, among other things, locomotive movements, postural movements as well as fulfilling, as a result of their significant participation in the whole body mass, a key role in the organism's metabolism<sup>2</sup>. Hence the correct functioning of the skeletal muscles enables the conducting of an active life style as well as preserving the organism's homeostatis<sup>3</sup>.

The skeletal muscle is a complex structure, into the composition of which enters: muscle fibre which fulfils a contractile function, motor fibre innervating the muscle fibre, blood vessels supplying the necessary nutritional components as well as connective tissue which integrates the mentioned elements into a functional whole<sup>1,4</sup>. The complex of these elements is essential both for the correct execution of movements as equally regenerative processes following muscle damage<sup>3</sup>.

The basic structural unit of the skeletal muscle is the spindle-shaped polynuclear muscle fibre. The number of fibres in the skeletal muscle is determined in the course of fetal development<sup>5</sup>. This means that in a mature muscle the number of muscle fibres does not undergo increase. This occurs as a result of the loss by their cell nuclei of the ability to divide.

However, the ability for DNA replication as well as mitotic divisions is at present possessed within the muscle tissue by a population of small mononuclear cells called satellite cells. These cells participate in the processes of the growth of skeletal muscles<sup>6</sup> as well as in the processes of muscle mass increase, i.e. the phenomenon of hypertropia<sup>5</sup>. Satellite cells are equally responsible for the processes of revival and regeneration of skeletal muscles both after intensive physical exertion<sup>7</sup> as equally after damage to the muscle tissue<sup>8,9</sup>.

### The localisation and morphology of satellite cells

Satellite cells were discovered by Mauro in 1961<sup>10</sup> in the skeletal mus-

cle of a frog. They were described on the basis of electro microscope photographs as cells lying on the surface of the muscle fibre between the sarcolemma and the basement membrane. At present it is known that satellite cells appear in all vertebrates occupying the self same anatomical position<sup>6,11,12</sup>. From 2 to 7 % of cell nuclei connected with a concrete muscle fibre belong to satellite cells<sup>5,12,13</sup>. The greatest concentration of satellite cells is found close to the motor plates and capillaries<sup>3,12,14</sup>. There equally exist differences in the location of satellite cells depending on the type of muscle fibre. The larger number accompanies the slowly contracting muscle fibres in comparison with quickly contracting muscle fibres<sup>14</sup>; besides the number of satellite cells reduces with age<sup>3</sup>.

Satellite cells in an undamaged muscle possess a small quantity of cytoplasm, in connection with which the shape of the cells takes on the shape of the nucleus<sup>10</sup>. The nuclei of satellite cells, in comparison to the nuclei of muscle fibres are smaller and contain a greater quantity of heterochromatin. This means that they are mitotically and metabolically inactive<sup>3,12,14,15</sup>. In response to muscle damage satellite cells remain active and their morphology changes. The number of cytoplasm increases while the number of heterochromatin decreases, cell organelle (Golgi apparatus, endoplasmic reticulum, ribosome, mitochondria) start to become visible<sup>12</sup>. The activated satellite cells, i.e. myoblasts, start to proliferate and subsequently link up with existing muscle fibres, or link up with themselves creating polynuclear myotubes, from which there subsequently arise muscle fibres<sup>14</sup>.

### The stages of skeletal muscle regeneration

Damage to skeletal muscles may result from mechanical injuries e.g. cuts, bruises, strain<sup>1</sup>. Other reasons for injury are: ischaemia, neurological disturbances, genetic diseases<sup>1,3</sup>. Regardless of the cause of injury the process of muscle repair consists of a determined sequence of events and runs its course in three phases<sup>4</sup>. The

first of which is the phase of deconstruction during which the damaged muscle fibres undergo necrosis, the environs of the injury start to be penetrated by cells in an inflammatory state. The subsequent phase is the phase of reconstruction which covers the phagocytosis of the necrotic fibre, the activation of the satellite cells, and the reconstruction of muscle fibres. In the course of this phase the creation of scar tissue is equally started, the revascularisation as well as reinnervation of the damaged place. The final phase is the phase of rebuilding, when the area covered by the scar tissue undergoes a reduction and the contractile functions of the muscle return.

### Activation of satellite cells

Satellite cells are activated by signals derived from the damaged muscle. These signals are factors of growth as well as cytokinins but their rich source are macrophages<sup>16,17</sup> infiltrating the region of damage and phagocytizing the necrotic material<sup>4,8</sup>. The macrophages free, among others, the fibroblast growth factor (FGF), the hepatocyte growth factor (HGF), the transforming growth factor beta (TGF $\beta$ ), the plate derivative growth factor (PDGF), the epidermal growth factor (EGF), the insulin derivative growth factor I (IGF-I), the insulin derivative growth factor II (IGF-II), the leukaemia inhibiting factor (LIF), interleukin 6 (IL-6)<sup>14,18</sup>. Growth factors are not only synthesized de novo. They may be equally produced by intact muscles and stored in intercell space, hence they are released as a result of rupture to tissue continuity<sup>4</sup>. In this way, in the place of tissue disturbance, there appear: HGF, FGF, LIF, TGF $\beta$ <sup>3,8,19</sup>, while their concentration depends on the extent of the damage<sup>3,14</sup>. Growth factors such as: HGH, FGF, TGF $\beta$ , IGF-I, IGF-II, not only activate the satellite cells but equally stimulate them to their own synthesis and secretion. Hence the mechanism of the influence of these factors on satellite cells is both paracrinic and autocrinic in nature<sup>3,14,20</sup>. Other substances of a stimulating action are: nitrogen oxide, factors released by motor neu-

rons. Nitrogen oxide indirectly participates in the activation of satellite cells resulting in the release of HGF from the inter-cell space<sup>12,21,22</sup>. Neuromediators and neurotrophic factors exert an activating effect<sup>14</sup>. Also muscle denervation leads to the activation of satellite cells<sup>14,19</sup>. Equally testosterone influences the processes of the regulation of satellite cell activity<sup>14</sup>. Male sex hormones control the growth of muscle mass. And so the greater the muscle mass of males than females in the case of mammals results from a greater average of muscle fibres<sup>5</sup>. The factors mentioned arouse the satellite cells from their state of slumber and stimulate them to commence cell divisions<sup>8,19</sup>. At the same time the majority of them impede the processes of differentiating myoblasts and ensure against premature fusion in the myotubes<sup>8,14,19</sup>. Growth factors also act as substances attracting satellite cells to the area of damage<sup>3,8,18,19</sup>. Besides which they have an influence on the processes of revascularization, the regeneration of nerve-muscular bonds as well as the reorganisation of the external-cell matrix<sup>3,19</sup>. As a result of injury the continuity of the basement membrane as well as of the cell membrane undergoes rupture, while the satellite cells are freed from their niche<sup>1</sup>. Under the influence of signals coming from the damaged environment they undergo activation. The activation of satellite cells does not only occur in the area of damage but over the entire length of the muscle fibre. This results in the migration of satellite cells to the area requiring regeneration<sup>3,14</sup>.

### The profile of gene expression in satellite cells during regeneration

Activated satellite cells are called myoblasts. Their subsequent participation in muscle regeneration is under the control of four genes belonging to the family of muscle-specific transcriptional factors. These genes are Myf5, MyoD, myogenina as well as Mrf4<sup>8,11,21</sup>. The sequence of the activation of these genes, and also the sequence of the appearance of transcripts of these genes occurs ac-

ording to a defined scheme of things<sup>21,23</sup>. Mitotically inactive satellite cells display expression of the Pax7 as well as Pax3 genes. After activation there appears the expression of the Myf5 gene responsible for the appearance of the proliferating potential of myoblasts. Next there is a fall in the level of the expression of the genes Pax7, Pax3 as well as Myf5, while the level of expression of the MyoD gene increases. The divisions of the myoblasts settle and the process of their differentiation begins. Myogenina is responsible for the final differentiation and subsequently the fusion of myoblasts and the creation of myotubules. When the muscle damage is little the differentiated myoblasts link up with the existing muscle fibres. When the damage is extensive there results a fusion of the myoblasts and the formation of new muscle fibres. The subsequent processes covering the maturity and hypertrophy of the regenerated muscle fibres depend on the activity of the Mrf4 gene. The newly formed muscle fibres are small and their cell nuclei are located in the central part of the fibre. The maturity of the regenerated muscle fibres will depend therefore on the increase by them of dimensions as well as the movement of the cell nuclei to the edges of the fibre<sup>3</sup>. So the regenerated muscle is morphologically and functionally indistinguishable from other undamaged muscles<sup>3</sup>.

### Self renewal of satellite cells

The characteristic feature of satellite cells is their ability to regenerate their own pool<sup>24</sup>. Without the ability for self regeneration the number of satellite cells would undergo a dramatic reduction and here not only as a result of their involvement in the process of regeneration. The constant mechanical activity of the skeletal muscles exposes them to sporadic periods of degeneration and regeneration during the whole life of an organism<sup>9</sup>. Even with the absence of serious damage during the physiological functioning of the muscles there occurs an exchange of cell nuclei in the fibres, for example in a rat 1-2% of nuclei undergo exchange

within the course of a week<sup>25</sup>. Hence without a supplementing of the stocks of satellite cells there would occur a depletion in their population.

The self regeneration of the population of satellite cells may come about as a result of an asymmetrical division<sup>12,15,26</sup>. Following activation the first division of a satellite cell leads to the creation of two different filial cells. One of these becomes the new satellite cell while the other divides further symmetrically generating in this way a pool of myoblasts<sup>15</sup>. It is postulated that the asymmetrical cell division is the result of non-random segregation of the template and filial threads of DNA remaining after replication during the cell division<sup>27,28</sup>. Both template threads are inherited through the filial cell which becomes a satellite cell. In this way its genes are protected from an accumulation of the mistakes that arose during replication<sup>27</sup>. The newly synthesized DNA threads are transmitted to a tissue determined filial cell<sup>27,28</sup>. The differentiated segregation of the DNA threads after replication can be connected with the regulation of gene activity in the filial cells<sup>8,27,29</sup>. Kuang et al.<sup>30</sup> have shown that the asymmetrical division of satellite cells in which there is present expression of the Pax7 gene and the lack of expression of the Myf5 gene, leads to the creation of cells of which the first displays, in a way similar to the parent cell, expression of the Pax7 gene as well as an absence of expression of the Myf5, while the second cell displays an expression of both the Pax7 and Myf5 gene. The first of these mentioned cells remains localised between the sarcolemma and the basement membrane while the second cell remains involved in the processes of myogenesis.

Another proposed mechanism enabling self regeneration of the satellite cell population is the withdrawal of myoblasts from the processes of differentiation. Following activation the satellite cells divide symmetrically<sup>12,15,26</sup>, while identical filial cells undergo a process of proliferation. Subsequently a part of the myoblasts in which there has been observed expression of muscle-specific transcriptional markers undergo differentia-

tion and take part in the reconstruction of the damaged fibres<sup>13</sup>. In a part of the myoblasts there occurs a fall in the activity of MyoD as well as myogenesis, during a simultaneous maintaining of Pax7 expression<sup>13</sup>. These cells withdraw themselves from the processes of differentiation, return to an inactive state and remain deposited beneath the basement membrane of the newly formed fibre<sup>24</sup>, in this way renewing the pool of satellite cells.

The model maintaining that the pool of satellite cells renews itself thanks to asymmetrical division as equally the model in which the self-regeneration process is possible thanks to symmetrical division are not mutually exclusive. Both models may jointly participate in maintaining the population of satellite cells in a state of dynamic balance<sup>8,12</sup>.

### Satellite cells and the regeneration of aging muscles

Aging is connected with a significant reduction in the mass, generated strength and endurance of skeletal muscles<sup>31</sup>. The loss in muscle mass results from a weakening in the efficiency to regenerate as a consequence of muscle damage<sup>14,32</sup>. The efficiency of muscle fibre regeneration is dependent on the number of available satellite cells<sup>33</sup>, their ability for proliferation<sup>34</sup> as well as the condition of the skeletal muscle microenvironment<sup>31</sup>. The total number of satellite cells reduces with age<sup>31,34,35</sup>. The most dramatic fall is observed in the postnatal period and is connected with intensive muscle growth<sup>3</sup>. Following the obtainment of sexual maturity the number of satellite cells continues to fall yet at a slower rate<sup>3</sup>. Along with progressive age there is observed an accumulation of fibres which do not contain satellite cells<sup>31</sup>. The decrease in the number of satellite cells connected with aging may be caused by the faulty regeneration of the pool of non-active satellite cells<sup>26,31,33</sup>. Proliferation is one of the key stages during regeneration, thanks to which an appropriate number of cell nuclei are supplied for the repaired or newly formed muscle fibres<sup>34,35</sup>. The ability to proliferate in

aging muscles may fall as a result of the involvement of satellite cells in the earlier stages of regeneration<sup>33</sup>. Satellite cells, in a way similar to other somatic cells, are capable of a limited number of cell divisions as every round of replication is connected with a reduction in telomers – the fragments of DNA from the ends of the chromosome<sup>35,36</sup>. Therefore the length of the telomers can reflect the history of cell divisions, as equally can determine the number of possible divisions before the cell loses the ability to replicate<sup>33,36</sup>. In vitro tests of biopsy fragments of skeletal muscles from healthy donors of various age did not show significant differences in the lengths of telomers between the group of young donors (20-28 years old) and the group of older donors (58-83 years old)<sup>33</sup>. Yet there was observed a significant fall in the length of telomers in the case of muscular dystrophy when the processes of degeneration and regeneration take place alternately after each other and require the constant inclusion of new cell nuclei to muscle fibres<sup>33</sup>. There was shown the existence of a weak correlation between the length of telomers and the ability for the proliferation of myoblasts derived from healthy donors at various ages (2-78 years old) despite the clear trend in the direction of a greater proliferation productivity in the case of longer telomers<sup>37</sup>. A significant fall in the length of telomers is rather connected with the recurrent occurrence of degenerative processes and may be a good indicator of pathological states<sup>33,34,35</sup>, while in healthy individuals the length of telomers maintains itself at a constant level over the course of a life and is sufficient for the satellite cells to maintain the ability for proliferation also in individuals of advanced years<sup>34</sup>. The likely reason for a lower regenerative ability in aging muscles is the weaker stimulation of the satellite cells through signals sent from the muscle microenvironment and not the limitation of their function<sup>32,38</sup>. Regardless of age, satellite cells preserve the ability to proliferate, differentiate as well as fuse in myotubes<sup>31,34</sup>. However, the tempo of these processes decreases in aging muscles, the myotubes created

are more delicate and there could be formed a scar hindering muscle functioning<sup>31,32,33,35</sup>. When contact between the myoblasts of aging muscles and that of younger individuals becomes possible, for example through muscle transplant<sup>39</sup> or also with factors present in the circulatory system thanks to the surgical anastomosis of these systems in two mice of different age<sup>38</sup>, there results an increased effectiveness of regeneration and the myotubes created are identical with those observed in younger individuals. A negative influence on muscle regeneration in aging individuals may be played by: a limited level of growth factors e.g. HGF, FGF, IGF<sup>14,31</sup>, a reduction in the number of macrophages<sup>14</sup>, disturbed paracrine interaction between the muscle fibre and the satellite cells connected with it<sup>31</sup>, a lower level of hormones circulating in the blood<sup>32</sup>, and also a reduction in the density of vascularization as well as changes connected with innervation of the muscle<sup>11,14,32</sup>. In summing up, deterioration in the conditions of the skeletal muscle microenvironment as well as a fall in the number of satellite cells are responsible for the weakened capacity for skeletal muscle regeneration during organism aging<sup>31</sup>.

### Therapeutic application of satellite cells

Satellite cells are perceived as material that may be used in cell transplantation in the case of illnesses which have at their basis impairment in the activity of muscle fibres, for example muscular dystrophy, myocardial insufficiency, defect in the functioning of the urethral sphincter<sup>40</sup>. Satellite cells possess a range of properties which enable their utilisation in cell therapies<sup>23</sup>. Satellite cells occupy a defined anatomical position consequently their localisation is easy. The taking of muscle biopsies is not a complicated procedure and enables one to obtain a huge number of satellite cells. Their molecular markers are known, for example the transcriptional factor Pax7 or the receptor c-met for HGF, thanks to which it is possible to isolate cells from the bioptic material by means

of flow cytometry. The parameters of cell culture are known, allowing for their efficient proliferation before transplantation. Thanks to acquaintance with the processes regulating activation, proliferation as well as the differentiation of satellite cells, it is possible to manipulate their behaviour both before as well as after transplantation. Besides which satellite cells are determined to create muscle tissue and consequently the risk of potential neoplasia after transplant is little<sup>40</sup>.

### Duchenne's muscular dystrophy

Muscular dystrophies are genetic diseases, often lethal, characterised by the gradual wasting of the muscles. The most common and best known is Duchene's muscular dystrophy. This is a severe hereditary disease recessive in nature, sex linked. It is the mutation of the gene localised on the short arm of the chromosome X in the Xp21 position that is responsible for the disease. This gene codes the dystrophin protein. Dystrophin enters into the composition of the complex of proteins linking the muscle fibre with the components of the external cell matrix, stabilising the sarcolemma during contraction. When there is a lack of dystrophin sarcolemma is not maintained under strain which results in damage to the fibres during muscle contraction. Regeneration of the fibres occurs thanks to the fusions of activated satellite cells which equally possess mutation in the dystrophin gene. The newly created fibres also undergo damage. Because the cycles of degeneration and regeneration constantly repeat themselves this leads to an exhaustion of the proliferation potential of the satellite cells and a sizeable reduction in their number. Fibres stop being reconstructed while the muscle fibre is replaced by connective and fat tissue, which suppresses the muscle functions. One of the therapy methods of Duchenne's muscular dystrophy is the utilisation of satellite cells revealing the correct expression of the dystrophy gene and derived from a foreign healthy donor. However, despite the promising results obtained in the case of animal

models, the clinical trails on people have been far from what was expected<sup>36,40</sup>. One of the reasons for lack of success is the immunological response of the recipient directed against alien myoblasts as equally hybrid muscle fibres that arise as a result of fusion with the implanted myoblasts<sup>5</sup>. Cells which avoid contact with antibodies display a limited ability to migrate from the place of injection<sup>5,23,36</sup>. The weak distribution of myoblasts requires repeated repetition of injection to ensure their appropriate density in the area of the muscle<sup>23,41</sup>. And multiple muscle injection is burdensome for the patient as well as requiring a significant quantity of injected cells<sup>23</sup>. Another method of treatment is the application of satellite cells isolated from the patient, which do not induce a defensive reaction in the organism; however, they require the genetic modification restoring the dystrophin expression<sup>36</sup>. The initial difficulty connected with this method is the construction of a suitable vector capable of holding such a large gene like dystrophin<sup>5,41</sup>. Another difficulty is the limited ability of satellite cells isolated from the patient with dystrophy to divide; connected with the progressing reduction in the length of telomers<sup>35,41</sup>. As a result only a limited number of cells are obtained from the patient, which during cultivation quickly lose their ability for division<sup>41</sup>. This limits the ability to obtain the respective number of myoblasts which can be genetically modified<sup>41</sup>.

### Myocardial failure

As a result of the weak distribution of satellite cells beyond the place of injection the possibility of their application in the case of localised damaged muscles is being sought<sup>40</sup>. Much hope is attached to the utilisation of satellite cells in the treatment of post-infarction heart failure. The majority of mature cardiomyocytes do not possess the ability for cell division, while the percentage of cells succumbing to mitosis is too small to effectively participate in the regeneration of the heart muscle<sup>7,42</sup>. Therefore also the cardiomyocytes dam-

aged during infarction are replaced first and foremost by connective tissue scar<sup>7,40,43</sup>. The post-infarction scar worsens the haemodynamic function of the heart and may lead to the remodelling of the left chamber as well as the appearance of aneurysm<sup>7,44</sup>. The aim of cell therapy is the replacement of the post-infarction area with contractile cells which restore the correct kinetics of the heart<sup>40,45</sup>. Satellite cells possess features which enable them to be used for transplants in the area of the post-infarction scar. First and foremost they are relatively insensitive to anoxia which increases their survival in the hostile environment of the post-infarction scar<sup>40,45</sup>. They may be isolated from a biopsy of the skeletal muscle of the very same individual, therefore the risk of transplant rejection is reduced<sup>43,45</sup>. Besides which the use of satellite cells in the regeneration of the heart muscle does not arouse doubts of an ethical nature<sup>44,45</sup>. Myoblasts transplanted to the heart are distinguished by multi-nuclear myotubes characteristic for the skeletal muscle<sup>45</sup>. Myotubes possess the ability to contract, they do not, however, create functional junctions with cardiomyocytes<sup>44,45</sup>. The active state between cardiomyocytes is conveyed by means of fissural junctions. Thanks to their presence integration is possible along with the spatial synchronisation of the contraction of the heart muscle<sup>40</sup>. It is considered that despite the absence of fissural junctions, the contraction of the skeletal fibres may be induced by their mechanical deformation at the moment of the contraction of neighbouring cardiomyocytes<sup>45</sup>. Another possible mechanism leading to the contraction of the skeletal fibres is dissemination, generated by contracting cardiomyocytes of action potential along the membrane<sup>45</sup>. The implanted cells can, in facilitating the functioning of the heart muscle, constitute an elastic scaffold which strengthens the wall of the left chamber and does not allow the spreading of the post-infarction scar<sup>46</sup>. One can equally not exclude the existence of paracrine interaction between the transplanted cells and the surround-

ing myocardium<sup>46</sup>. Satellite cells may be implanted in the region of the post-infarction scar during cardio-surgery or also introduced percutaneously to coronary veins by means of catheters<sup>40,44</sup>. Clinical tests have shown that as a result of the injection of satellite cells there is created a functional, new, contractile tissue and subsequently an improvement in the ejection fraction of the heart<sup>44,47</sup>.

### Defect of the extrinsic urethral sphincter

Grafts of satellite cells may be equally used in the treatment of urinary incontinence against the background of a defect in the extrinsic urethral sphincter<sup>40</sup>. The frequency of urinary incontinence increases with age<sup>48</sup>. In women the causes of this condition are hormonal changes during menopause, vaginal birth as well as urino-gynaecological operations<sup>49,50</sup>. In men urinary incontinence is usually the side effect of surgery on the prostate gland arising as a result of cutting the muscle fibres of the sphincter<sup>50,51</sup>. In order to strengthen the weakened sphincter structure there is applied the periurethral injection of various substances, e.g. collagen, autologous fat, silicon microimplants<sup>49</sup>. However this method is relatively low in effectiveness, for the materials obtained have a tendency to migrate beyond the area of application as well as also absorption or disintegration<sup>49</sup>. An autologous graft of satellite cells gives the possibility of improving the sphincter thanks to the introduction of new, normally functioning tissue<sup>48</sup>. The sphincter is not a large structure therefore equally its treatment requires a small number of satellite cells<sup>50</sup>. They can potentially ensure good anatomical integrity<sup>40</sup> as the extrinsic urethral sphincter is composed of transversely striated fibres. Besides which the extrinsic urethral sphincter also possesses an internal population of satellite cells which display expression of the Pax7 gene and after activation Myf5 as well as MyoD<sup>51</sup>. Thus the autologous grafting of satellite cells may imitate the natural process of the regeneration of the defective sphincter<sup>49</sup>. Tests on animal models have shown

that satellite cells injected into the sphincter survive and are capable of differentiating into muscle fibres or of integrating with the host fibres as well as causing an increase in the imperviousness of the urethral sphincter<sup>48,49</sup>. Clinical attempts confirm the contribution of grafted autologous satellite cells in the reconstruction of the muscle tissue of the extrinsic urethral sphincter<sup>52,53</sup>. The injection of cells causes an increased thickness in the sphincter, improving its contractibility<sup>52,53</sup>, which leads to a better control over urination in patients with urinary incontinence<sup>49</sup>.

### Stem cells

Satellite cells are the main, though not the only, source of cells with a regenerative potential. There are also present in skeletal muscles subpopulations of mesodermal stem cells which are myogenic in nature. These include: side-population muscle cells, mesoangioblasts as well as pericytes<sup>54,55,56</sup>. Side-population muscle cells are localised between muscle fibres and are characterised by a lack of accumulation of the Hoechst fluorescent pigment<sup>54,57</sup>. Mesoangioblasts and pericytes are cells connected with the walls of blood vessels<sup>55,56,58</sup>. The mentioned subpopulations of cells possess the ability to differentiate in a myogenic direction and successfully participate in muscle regeneration<sup>3,11,21,56,59</sup>. In *in vitro* conditions they differ in multinuclear myotubule but only during joint cultivation with the myoblast cell line<sup>56,60,61</sup>. Autologous cells may be used in tests on living organisms, which eliminates the complications connected with the immunological response as well as the risk of rejection of the transplanted cells<sup>55,59</sup>. As opposed to satellite cells they are able to breach blood vessel walls hence they can also be introduced to the organism intra-arterially and not by means of cumbersome muscular injection<sup>55,56,59</sup>. In the area of the damaged muscle they form new muscle fibre, as equally possessing the possibility to renew the satellite cell pool<sup>56,60,62</sup>. The negative effects of the therapeutic application of myogenic stem cells include: the risk of

embolism e.g. in the lungs, heart and brain<sup>55</sup>. Through wandering through the blood vessels the cells can miss the intended muscle<sup>59</sup>. As they are poorly differentiated cells they can be stimulated in the organism to transform into other forms of tissue than muscle<sup>59</sup>.

In physiological conditions satellite cells are responsible first and foremost for the process of regeneration, while the myogenic stem cells play rather an insignificant role<sup>11,59</sup>. However, in the case of extensive damage, myopathy as well as aging muscles - when this stock of satellite cells undergoes exhaustion<sup>3,11,59</sup>, the transplanted stem cells are able to take on their functions and differentiate in a myogenic direction<sup>59</sup>. They constitute therefore a promising source of cells which may be utilised, for example, in the treatment of muscular dystrophies<sup>55,56,58,59</sup>. Further research is, however, essential before myogenic stem cells are used in clinical tests<sup>63</sup>.

### Conclusion

Skeletal muscles are characterised by a high regenerative productivity, for which the small population of satellite cells is responsible. Numerous experimental tests have allowed one to become acquainted with the factors regulating their life cycle, beginning from an inactive state through activation for cell division, and finishing with differentiation into myotubes and the reconstruction of muscle fibres. Thanks to the ability of satellite cells to self-renew it is possible to maintain their pool despite the repeating episodes in the course of the whole life of an individual of degeneration and regeneration of the skeletal muscles. The fall in their number that is connected with organism aging, observed also in muscular dystrophies, is the reason for the limited possibilities of regenerative muscles. Satellite cells are considered to be potential material for cell grafting which would enable the correct functioning of impaired muscles. This is spoken for by their availability and the minimal risk of potential neoplasia following grafting. Further research into the therapeutic effective-

ness of satellite cells will more than likely concentrate on establishing the procedures for increasing their survival rate after grafting as well as defining the longevity of the effects of such operations<sup>40</sup>.

The work was carried out within the framework of the Medical University in Białystok Grant no 3-18719

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*Translated from the Polish by Guy Torr*