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A prosurvival and proangiogenic stem cell delivery system to promote ischemic limb regeneration

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ABSTRACT

Stem cell therapy is one of the most promising strategies to restore blood perfusion and promote muscle regeneration in ischemic limbs. Yet its therapeutic efficacy remains low owing to the inferior cell survival under the low oxygen and nutrient environment of the injured limbs. To increase therapeutic efficacy, high rates of both short- and long-term cell survival are essential, which current approaches do not support. In this work, we hypothesized that a high rate of short-term cell survival can be achieved by introducing a prosurvival environment into the stem cell delivery system to enhance cell survival before vascularization is established; and that a high rate of long-term cell survival can be attained by building a proangiogenic environment in the system to quickly vascularize the limbs. The system was based on a biodegradable and thermosensitive poly(N-Isopropylacrylamide)-based hydrogel, a prosurvival and proangiogenic growth factor bFGF, and bone marrow-derived mesenchymal stem cells (MSCs). bFGF can be continuously released from the system for 4 weeks. The released bFGF significantly improved MSC survival and paracrine effects under low nutrient and oxygen conditions (0% FBS and 1% O_2) in vitro. The prosurvival effect of the bFGF on MSCs was resulted from activating cell Kruppel-like factor 4 (KLF4) pathway. When transplanted into the ischemic limbs, the system dramatically improved MSC survival. Some of the engrafted cells were differentiated into skeletal muscle and endothelial cells, respectively. The system also promoted the proliferation of host cells. After only 2 weeks of implantation, tissue blood perfusion was completely recovered; and after 4 weeks, the muscle fiber diameter was restored similarly to that of the normal limbs. These pronounced results demonstrate that the developed stem cell delivery system has a potential for ischemic limb regeneration.

Statement of significance

Stem cell therapy is a promising strategy to restore blood perfusion and promote muscle regeneration in ischemic limbs. Yet its therapeutic efficacy remains low owing to the inferior cell survival under the ischemic environment of the injured limbs. To increase therapeutic efficacy, high rate of cell survival is essential, which current approaches do not support. In this work, we tested the hypothesis that a stem cell delivery system that can continuously release a prosurvival and proangiogenic growth factor will promote high rates of cell survival in the ischemic limbs. The prosurvival effect could augment cell survival before vascularization is established, while the proangiogenic effect could stimulate quick angiogenesis to achieve long-term cell survival. Meanwhile, the differentiation of stem cells into endothelial and myogenic lineages, and cell paracrine effects will enhance vascularization and muscle regeneration.

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1. Introductions

Atherosclerotic peripheral artery disease (PAD) affects more than 27 million people in North America and Europe [1,2] PAD decreases blood perfusion in the tissues and causes tissue ischemia. Critical limb ischemia (CLI) represents the most severe form of PAD. It is characterized by low blood perfusion, severe tissue ischemia, and degenerated skeletal muscle. Quick restoration of blood perfusion to salvage existing cells and promotion of muscle repair represent the optimal goals for CLI treatment [1,3–8] However, current surgical procedures show limited efficacy [1,8–10]

To improve therapeutic efficacy, approaches like drug delivery [11] and stem cell therapy [3,12–19] have been explored. The former involves delivery of growth factors that either stimulate vascularization or promote muscle regeneration into the limbs. For example, VEGF, PDGFBB and bFGF have been used for vascularization [20–22], while IGF-1 has been employed for muscle regeneration [23,24]. Different studies have demonstrated that delivery of two or more types of growth factors with different functions can result in a greater therapeutic efficacy than delivery of single growth factors [11]. Efficacy of the drug delivery approach is dependent on the amount of bioactive drugs released to the ischemic limb, duration of the drug release, and sequence of the release. Overall, current drug delivery approach shows limited success due to the rapid release of delivered drugs, loss of bioactivity, inadequate amount of drug released to the tissue, and inappropriate drug gradients and release sequence [11]. Cell therapy is an alternative approach. It includes direct injection of stem cells into the tissue or encapsulating cells in the constructs followed by injection [1,15,25]. The transplanted stem cells serve two purposes: differentiation to replace damaged host cells, and providing paracrine effects for vascularization and muscle repair.

Various stem cell types including embryonic stem cells (ESCs) [26], mesenchymal stem cells (MSCs) [14,15,25,27,28], adipose tissue-derived cells [29–32], cell lines selected based on the presence of specific markers [33,34], and induced pluripotent stem cells (iPSCs) [16] have been used for ischemic limb regeneration. Among them, MSCs stand out because of their ability to differentiate into myogenic lineages for muscle regeneration, and into endothelial cells for vascularization [35,36]. MSCs also provide paracrine effects for ischemic limb regeneration. Among the number of different growth factors they secrete, IGF-1 promotes myoblast proliferation and satellite cell differentiation [11,37,38], and bFGF, VEGF and PDGFBB stimulate angiogenesis. Therefore, MSCs have the potential to induce not only vascularization, but also muscle repair in the ischemic limb through direct and indirect effects.

Various animal studies have demonstrated that stem cell therapy could, to some extent, improve blood perfusion in the ischemic limb and even promote muscle repair in some cases [1,39,40]. However, clinical trials have shown only a transient therapeutic benefit [1,41]. Overall, current stem cell therapy has a low efficacy in improving blood perfusion and muscle repair [1,41] One of the key causes is the poor survival rates of transplanted cells [1,40–42]. Several studies have demonstrated that only ~20% of cells remained in the tissue 24 h after transplantation, and only ~3% remained after 30 days [42–45]. Among the possible triggers for cell death, such as ischemia, immune response, inflammation and oxidative stress, ischemia represents the most significant problem to be solved [1,40–42].

Promoting high rates of both short- and long-term cell survival under ischemic conditions is one of the key steps to significantly increase therapeutic efficacy of stem cell therapy for CLI [8]. To improve cell survival under ischemia, approaches have been used including blocking the apoptotic signaling pathways of cells [46,47], preconditioning cells before transplantation [48,49], co-transplanting with cells rich in paracrine effects [50,51], using prosurvival cocktails [52], promoting angiogenesis [50], and co-transplanting with hydrogels [53–56]. While these approaches increased cell survival to an extent, achieving high rates of both short- and long-term cell survival remains challenging and insignificantly addressed. Those approaches that induce endogenous cellular survival mechanisms, co-transplant stem cells with other cells, and use prosurvival cocktails, can only temporarily improve cell survival, as adequate vascularization cannot be readily achieved to relieve ischemia for long-term cell survival. The approaches focusing on promoting angiogenesis show significant cell death before angiogenesis can occur. While using hydrogels, such as collagen and hyaluronic acid, can decrease cell apoptosis, vascularization around matrices for long-term cell survival is challenging [53–56].

In this study, we hypothesized that a stem cell delivery system that can continuously release a prosurvival and proangiogenic growth factor will promote both short- and long-term cell survival in the ischemic limbs. The prosurvival effect could promote cell survival before vascularization is established, while the proangiogenic effect could stimulate quick angiogenesis to promote long-term cell survival. Meanwhile, the differentiation of MSCs into endothelial and myogenic lineages, and MSC paracrine effects will enhance vascularization and muscle regeneration. bFGF was used due to its prosurvival and proangiogenic effects [22,57–62]. We investigated how the controlled release of bFGF affected MSC survival and paracrine effects in vitro under low nutrient and oxygen conditions, and how the delivery system enhanced MSC survival and differentiation, muscle regeneration and blood perfusion recovery in ischemic limbs.

2. Experimental materials and methods

2.1. Materials

All chemicals were purchased from Sigma–Aldrich unless otherwise stated. 2-hydroxyethyl methylmethacrylate (HEMA) was purchased from TCI and passed through an inhibitor remover column to eliminate inhibitor. N-isopropylacrylamide (NIPAAm, Alfa Aesar) was purified by recrystallization for 3 times using hexane. 3,6-Dimethyl-1,4-dioxane-2,5-dione, acryloyl chloride, sodium methoxide and chondroitin sulfate were used as received.

2.2. Hydrogel synthesis

The hydrogel was synthesized from NIPAAm, HEMA and a macromer based on acrylic acid and oligolactide (AA-oligoLA). The macromer was synthesized by a two-step method [63]. In the first step, OligoLA was generated by ring-opening polymerization of lactide using $NaOCH_3$ as an initiator. In brief, 50 g (0.347 mol) of D,L-lactide was dissolved in 100 mL of CH_2Cl_2 and charged into a one-necked flask. One gram (0.019 mol) of $NaOCH_3$ dissolved in methanol was then added. The reaction was conducted at 0 °C for 2 h. The mixture was neutralized with 0.1 M HCl, and washed with DI water. The oligoLA was obtained by evaporating the organic layer at 60 °C. Its structure was confirmed by ^1H NMR. The average number of LA units in each oligomer was 2.5. In the second step, oligoLA was esterified using acryloyl chloride. The oligoLA (32.4 g, 151.3 mmol) was dissolved in 100 mL of CH_2Cl_2. Triethylamine triethylamine (23.6 mL, 169 mmol) was then added. After the solution was cooled down to 0 °C in an ice bath, acryloyl chloride (13.6 mL, 169 mol) was added dropwise for 1 h. The mixture was stirred overnight at room temperature, then rinsed with 0.2 M Na_2CO_3, 0.1 M HCl and DI water in sequence. The final product AA-oligoLA was obtained by