Functional Muscle Regeneration by Delivery of Mesenchymal Stem Cells and Basic Fibroblast Growth Factor

Yanyi Xu[1], Minghuan Fu[1], Zhihong Li[1], Xiaofei Li[1], Zhaobo Fan[1], Peter Anderson[1], Zhenguo Liu[2], Jianjun Guan[1]

1. Department of Materials Science and Engineering, Ohio State University.
2. Heart and Lung Institute, Ohio State University.

Statement of Purpose: Severe injuries, tissue ischemia and genetic defects impair skeletal muscle regeneration. Delivery of stem cells capable of differentiating into skeletal muscle cells may facilitate the regeneration [1-3]. However, ischemic environment in the injured muscles significantly comprises the therapeutic efficacy, as cell survival and differentiation are limited in such environment. Using biomaterials as cell delivery vehicles may address this issue. In this report, we demonstrated that using hydrogels with angiogenesis factor basic fibroblast growth factor (bFGF) significantly augmented cell survival and differentiation under ischemic conditions both in vitro and in vivo.

Methods: Hydrogel was synthesized by free radical polymerization using N-isopropylacrylamide, 2-hydroxyethyl methacrylate, and macromer Acrylic acid-Polylactide (APLA). Basic fibroblast growth factor (bFGF) was purchased from Life Technology and used as received. For in vitro study, MSCs (10 million/mL) were encapsulated in the hydrogel containing bFGF (50μg/mL) and heparin (1 mg/mL). The cells were cultured under hypoxic condition (1% O_2, 5% CO_2, 0% FBS) for a 2-week period. Cells seeded in the hydrogel without bFGF under normal and hypoxic culture conditon were used as control. For in vivo study, hindlimb ischemia was first induced in mice (aged 8 to 10 weeks) by permanent ligation of femoral artery and vein. A total volume of 200 μl mixture was then injected into quadriceps femoris muscles by 4 injection points in between the ligation points. Treatment groups included gel-only, gel+cells and gel+cells+bFGF. Open surgery mice were used as negative control and non-operated mice as positive control.

Results: The hydrogel solution was thermo-sensitive with a gel-sol transition temperature (GSTT) around 26°C. It can be readily injected through a 26G needle. The hydrogel exhibited a Young's modulus of 17.1±3.4 kPa at

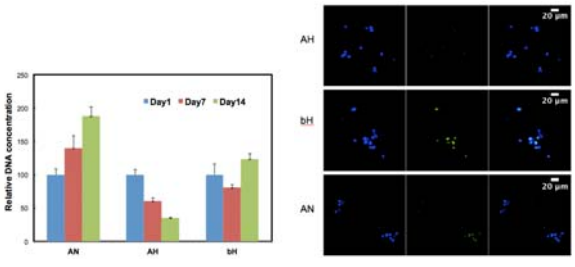

Figure 1. (a) dsDNA content (b) MHC protein expression (Nucleus-blue, MHC-green, merge) of MSCs seeded in gel during a 2-week in vitro culture period. AN: MSCs in gel under normal condition. AH: MSCs in gel under hypoxic condition. bH: MSCs in gel with bFGF under hypoxic condition.

37ºC (10% w/v in DPBS). Its degradation product has a GSTT above 37 ºC, so that it is soluble in body fluid. The hydrogel was able to gradually release the encapsulated bFGF for two weeks. And the released bFGF was bioactive. Cell survival was significantly improved with bFGF release during the 2-week in vitro culture period under ischemic conditions (Figure 1a). MSCs differentiated into skeletal muscle cells in the hydrogel with bFGF release while did not differentiate in the hydrogel without bFGF release based on gene expressions and protein expression (Figure 1b) results. In vivo, 4 weeks after implantation, MSCs encapsulated in the hydrogel showed a high expression of myogenic marker MHC (Figure 2), suggesting their successful differentiation into skeletal muscle cells. In addition, muscle blood perfusion (Figure 3) was also significantly improved by delivering MSCs with bFGF release, resulting from the increase of blood vessel density in the injured muscles according to vWF staining result. The blood perfusion is even recovered to normal level 4 weeks after surgery.

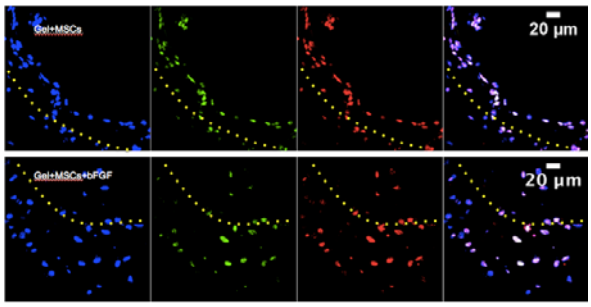

Figure 2. MHC protein expression (Nucleus-blue, MHC-green, live cell tracker CM-Dil-red and merge) of transplanted MSCs in gel without/with bFGF 4 weeks after injection in vivo.

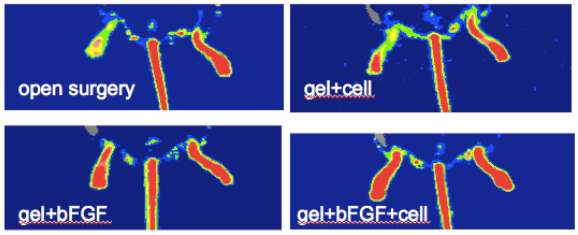

Figure 3. Blood flow (laser Doppler) 4 weeks after surgery.

Conclusions:

Thermo-sensitive and biodegradable hydrogel with skeletal muscle tissue-like matrix modulus (17.1±3.4 kPa) was successfully synthesized and used to deliver MSCs. By adding basic fibroblast growth factor, encapsulated MSCs were able to survive and differentiate into mature skeletal muscle cells both in vitro and in vivo.

References:

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