A study on use of prolotherapy as pharmacopuncture to augment fascia proliferation and repair

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Background & Aim :

As in the case of fibromyalgia, disorder of the fascial continuum has great impact On emotion. Receptors (as Ruffini-, Golgi- and Pacini-type endings) within fascia sense “fascia tear”, and relay signals by proprioception or interoception to emotion related frontal cingulate cortex and insula. Healing fascia tear and restore continuum is fundamental to promote mental health. Prolotherapy is a kind of regenerative medicine which involved injecting high concentration of D-glucose (dextrose) to strengthen injured fascia tissue (or acupoint in pharmcoacupuncture). It is not sure about the cellular mechanism of prolotherapy.

Keywords : Prolotherapy, Pharmcoacupuncture, Myofibroblast Transformation

Materials & Methods :

In our study, NIH-3T3 fibroblast cell line was used and maintained at 37°C with 5% CO2 in an air atmosphere; the cells were grown in DMEM low glucose (5nM) with 10% bovine calf serum. At each experimental time point, either high glucose (25nM) DMEM or low glucose (5nM) DMEM will be substituted with original growth medium. MTT assay was used to measure cell proliferation ability and insert induced migrating assay to test migration ability. By measuring cell proliferative and migration ability we can verify effect of high glucose stimulation. Furthermore, we use western blot to verify if myofibroblast transformation took place by testing expression change of α-smooth muscle actine (α-SMA) and type I collagen.

RESULT:

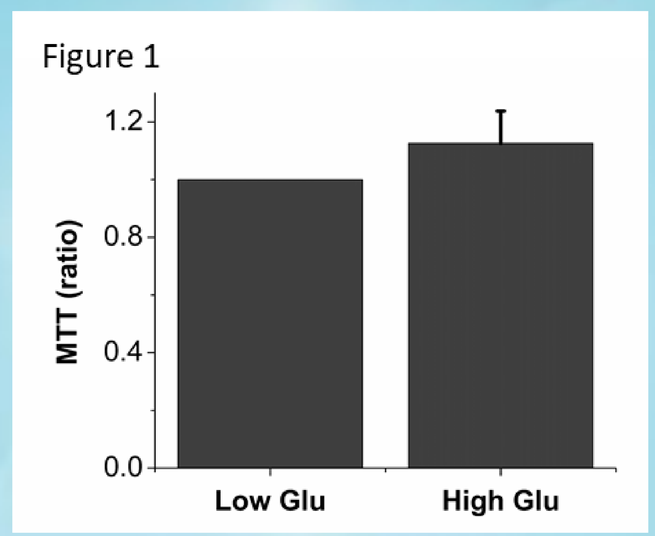

Figure 1: High glucose stimulation (25nM) stimulate proliferation of 3T3 fibroblast as compared to low glucose (5nM). Cells were seeded to 96 well and incubated for 24 hr either in high or low glucose medium. Absorption of 6 wells were averaged for one data measure. Relative value was used for statistic analysis as low glucose value for each series was set as 1. High glucose induced fibroblast proliferation as compare to low glucose (1.13 ± 0.19 fold, significancy not meet yet with number needed to be added n = 3 per group).

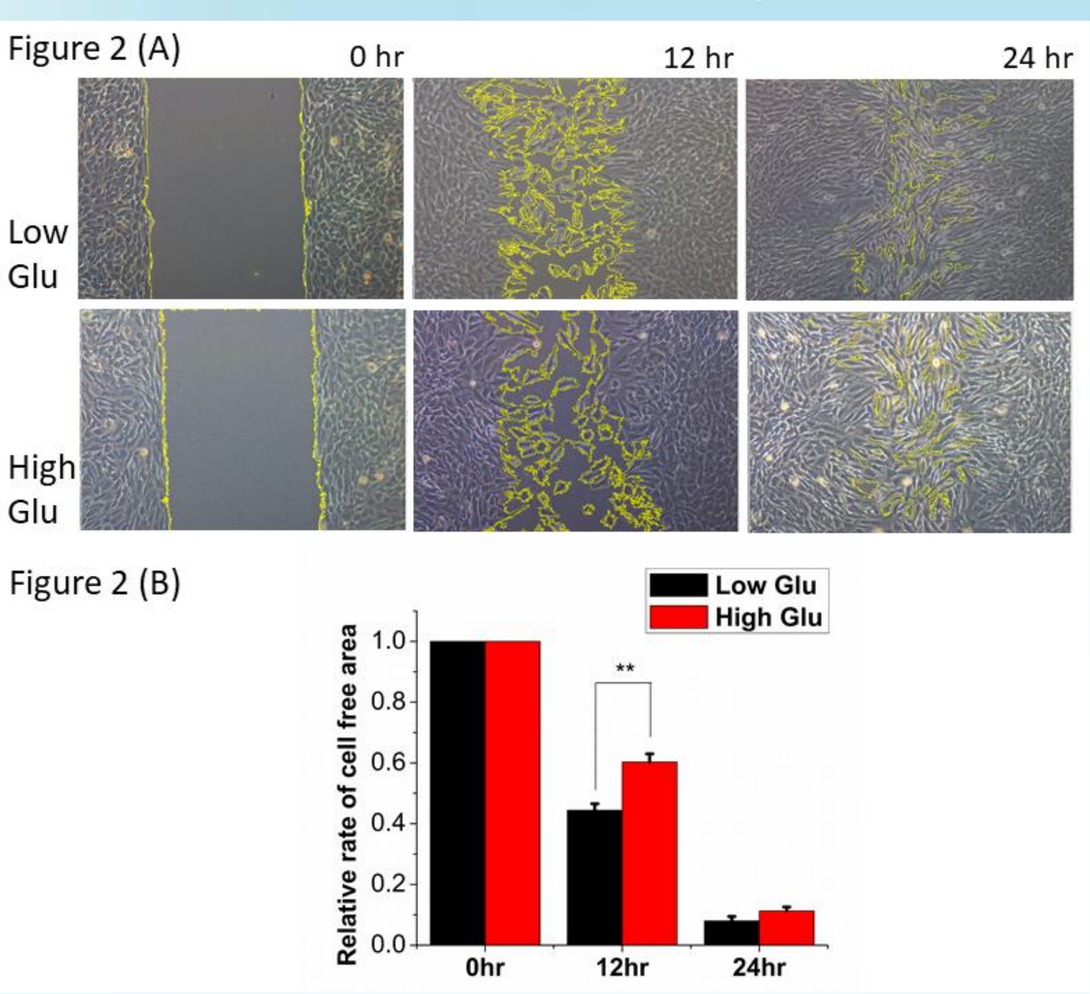

Figure 2: High glucose stimulation inhibit fibroblast migration as shown by migration assay. Ibidi insert was used to create gap and then removed. After removal of insert, either high or low glucose medium. Images chage of the gaps were captured at 0hr, 12hr and 24hr. Cell-free area was measured using ImageJ. Relative value was used for analysis and area at 0hr was set at one. Significant difference between high and low glucose stimulation was found at 12hr (0.60 ± 0.10 and 0.44 ± 0.07, P<0.01) but not seemed at 24hr (0.11 ± 0.05 and 0.08 ± 0.05, P= 0.11). Statistical significance ($p < 0.05$) between groups was determined with t test. (n=10-15)

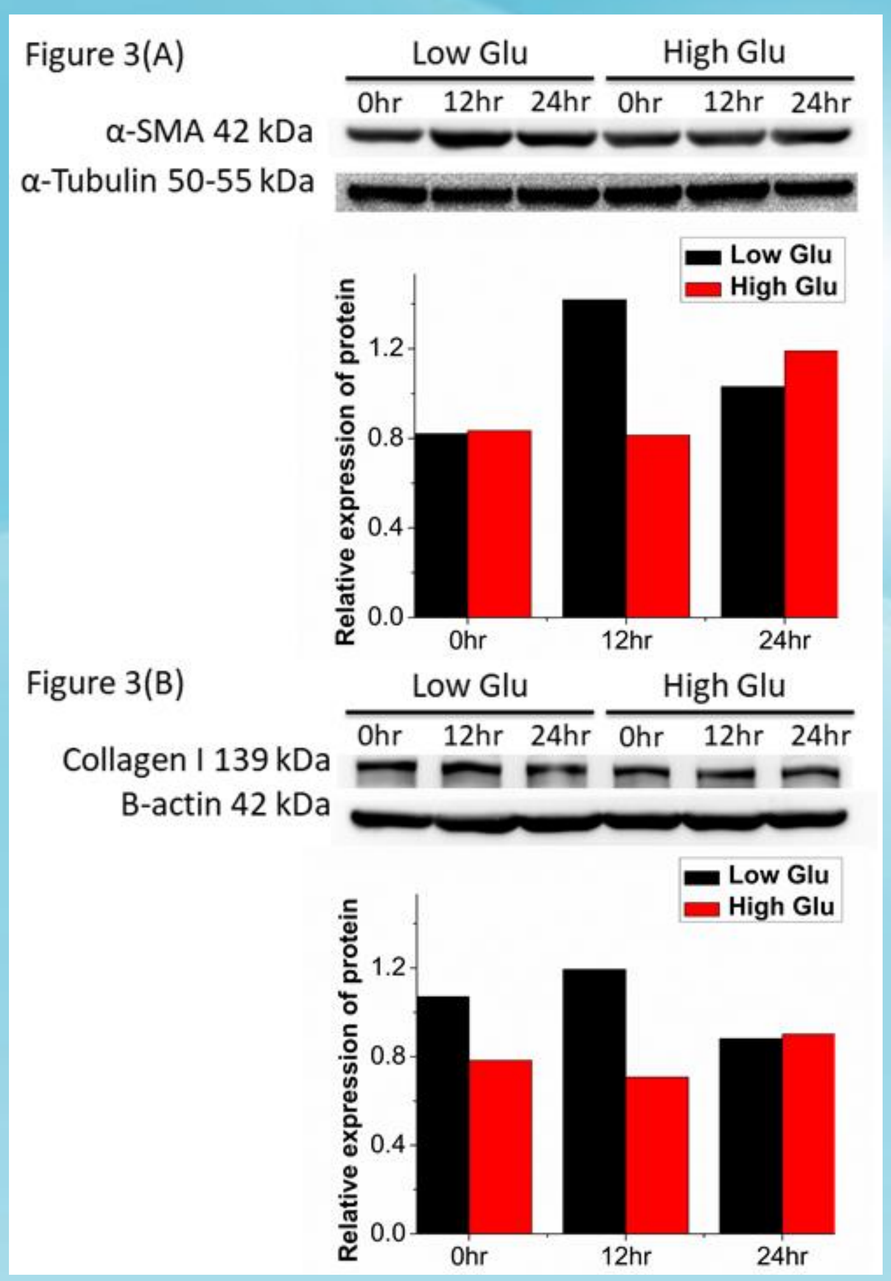

Figure 3: High glucose stimulation myofibroblast transformation and collagen type I production. Cells were collected and lysed after stimulation by high or low glucose medium at 0hr, 12hr, and 24hr. For current data, transient increasement of α-SMA (1.42 and 0.81 fold to internal control) and collagen (1.19 and 0.71 to internal control) was found at 12hr after low glucose stimulation. At 24hr response and increase after high glucose stimulation for α-SMA (1.03 and 1.19 fold to internal control) but not collagen (0.88 and 0.90 to internal control).

Conclusion:

Our results suggested that stimulation with high glucose will bring proliferative effect to NIH-3T3 fibroblast. Explaining the effect of prolotherapy or glucose pharmcoacupuncture. However, increasement of glucose would impair fibroblast migration. This may imply that clinical use of prolotherapy may need to use glucose concentration above physiological concentration but as low as possible. This would bring the most optimal result considering fascia tear repair.

As seemed in our western blot, eventually by 24 hr α-SMA increased expression, meaning that myofibroblast transformation took please by 24 hr. As myofibroblast bears the ability to contract and increase fascia tension, this may imply that myofibroblast transformation to increase fascia tension and restore biotensegrity in weakened fascia tissue may be another possible mechanism of prolotherapy.

Reference:

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