Accepted Manuscript

Muscle stem cell intramuscular delivery within hyaluronan methylcellulose improves engraftment efficiency and dispersion

Sadegh Davoudi, Chih-Ying Chin, Michael C. Cooke, Roger Y. Tam, Molly S. Shoichet, Penney M. Gilbert

PII: S0142-9612(18)30312-0

DOI: 10.1016/j.biomaterials.2018.04.048

Reference: JBMT 18634

To appear in: *Biomaterials*

Received Date: 1 January 2018

Revised Date: 21 March 2018

Accepted Date: 24 April 2018

Please cite this article as: Davoudi S, Chin C-Y, Cooke MC, Tam RY, Shoichet MS, Gilbert PM, Muscle stem cell intramuscular delivery within hyaluronan methylcellulose improves engraftment efficiency and dispersion, *Biomaterials* (2018), doi: 10.1016/j.biomaterials.2018.04.048.

General Response to Reviews

We are thankful for the careful review of our manuscript to Biomaterials entitled 'Muscle stem cell intramuscular delivery within hyaluronan methylcellulose improves engraftment efficiency and dispersion'. We have addressed all of the reviewers concerns and hope that the manuscript is now suitable for publication in Biomaterials.

Detailed Point-by-Point Response to Reviewers

Reviewer #2: Isolate mononucleated cells from murine or human skeletal muscles possess stem cell properties. Muscle stem cell transplantation provided hope for diseased skeletal muscle therapy. Except for expanding muscle stem cells ex vivo, optimizing the transplantation procedure to maximize engraftment efficiency is another bottleneck of the development of relevant clinical therapy. By delivering the therapeutic cell population blended with hyaluronan and methylcellulose saline, authors tried to improve transplantation engraftment efficiency and graft cellular dispersion of injected intramuscular muscle stem cell.

1. Authors had found the phenomenon of that there are 45% (GFP+) fibers 4 weeks after 5×103 or 10×103 GFP+ MuSCs intramuscular transplanted within HAMC than that in saline control, which indicates HAMC can improve engraftment efficiency and dispersion. But unfortunately, the result of HAMC improving muscle stem cell ejection efficiency cannot support these benefits of delivering MuSCs within HAMC.

We absolutely agree with the reviewer that the 6% effect noted in vitro is not a likely contributor to the in vivo findings. As such, in the Results section we state the statistically significant 6% difference in cell retrieval, but do not indicate that this finding explains the in vivo results. Instead, in the Discussion we are careful to point out exactly the caveat you indicate. Specifically, we state "..experimental error introduced during the process of tissue digestion, mononucleate cell retrieval, and flow cytometic analysis, may introduce error that effectively masks the small, but statistically 6% difference in transplanted cell number". However, we agree that an important take-home point of this result is that in vitro findings should be taken with a grain of salt. We have now extended this point to our Discussion.

2. Both the CD44KO MuSCs and wild type MuSCs will grow quicker in HAMC is not enough to conclude that HAMC promotes MuSC proliferation via a CD44-independent mechanism. By the way, this is not relative mechanism to explain the inside mechanism of this manuscript.

CD44 is the most commonly studied receptor of HA, and several studies showed that CD44-HA interactions drive cell proliferation, as we cited in our Introduction. As such, we sought to determine whether this receptor-ligand interaction could explain why we observed more MuSCs incorporating EdU when cultured in HAMC. The reviewer rightly points out that we find that regardless of the presence of CD44, a higher proportion of MuSCs incorporate EdU when cultured in the context of HAMC. This was surprising as we were expecting to find that the CD44 KO MuSCs in HAMC would exhibit a reduction in the proportion of cells that incorporate EdU. As such, we are forced to conclude that the CD44 receptor does not play a role in HA-mediated effects on MuSC cell cycle entry. This is interesting, because it suggests that another HA-receptor, such as RHAMM, might instead be responsible, though teasing this out is beyond the scope of the current work.