Reviewer’s report

Title: A new web-based method for automated analysis of muscle histology

Version: 2 Date: 9 November 2012

Reviewer: Hannah Radley-Crabb

Reviewer’s report:

This is a concisely written introduction to a new scientific analysis platform – MyoScan and its potential application to the histological analysis of dystrophic skeletal muscle. I believe that this analysis platform has the potential to be well received by the research community.

Major compulsory revisions:

My concern with this manuscript is that the authors have not conducted their own manual analysis of their skeletal muscle sections and have simply referred the results generated by MyoScan to published literature from 1993. While I agree that the numbers produced by MyoScan seem reliable and certainly fit with the expected pathology of mdx mice at that age, I feel it is essential that the authors manually analyse their own sections (perhaps n=4) for both Feret’s diameter and centrally nucleated myofibres and compare their data directly to that generated by MyoScan. It is important to know this direct correlation as a ‘proof-of-concept’ for MyoScan. For example there is a 10% difference in number of centrally nucleated myofibres in the soleus of mdx mice generated between MyoScan and the 1993 publication – clarification is needed.

Methods, 2.2 last paragraph - was the whole muscle CSA imaged and analysed by MyoScan? If not how was the field of view chosen and approximately how much of each muscle was analysed? Analysis of the whole muscle cross sectional area is the most appropriate method and leads to less biased results.

How does MyoScan cope with histological imperfections such as folds, freeze fracture, uneven or pale staining? Please comment somewhere in the manuscript.

Does WGA need to be used? It seems that any membrane / extracellular matrix marker could be used. Please comment somewhere in the manuscript.

It is unclear why MHC double staining is explained in such detail in the methods section – and then no data is presented in the results? It is probably more suitable just to address MHC staining in the discussion (page 18/19).

Results 3.3 first paragraph - please provide a reference for the statement that “…. Feret’s diameter is the most robust parameter in order to measure muscle fibre size…. ” I presume this is referring to the other parameters listed in the TreatNMD SOP?
Minor essential revisions:

Introduction, paragraph 4 – Please provide the link to the TreatNMD website SOPS (this is useful for new students, new researchers etc. who are unfamiliar with the website).

Methods, 2.2 last paragraph – Please provide some further information on the associated costs, registration process and international accessibility of MyoScan.

Methods, 2.2 last paragraph – Please provide some further information on file size, type and maximum upload.

Minor issues not for publication:

Abstract, line 4. “…improve muscle strength and quality of life…”

Abstract – conclusion. “Automated analysis of histological parameters….”

Introduction, paragraph 2. “…muscle is characterised by infiltrating…” Correct to British spelling.

Introduction, paragraph 6. change both histologic to ‘histological’

Results, paragraph 1. Correct spelling of ‘diaphragm’.

Discussion, paragraph 2. “…literature [11, 12]. C57BL/10 mice showed hardly any internally nucleated…” Full stop required, insert ‘any’.

Discussion, paragraph 2. “…fibre sizes [12]. Since we…” Space required after reference.

Figure 3 – ‘Figure 3’ label on graph is inappropriately placed and covers up the axis label.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare I have no competing interests.