Peer-review Report
Short Notes applies an anonymous, free-of-charge peer-review process conducted by experts in the relevant field. After acceptance, reviewers' reports, authors' responses and revisions, and the editorial decision are published, and reviewers may choose to disclose their identity.

Peer-reviewers:

- Reviewer 1 : choose not to disclose their identity
- Reviewer 2 : choose not to disclose their identity

Executive Editor: Marc Le Bert, FC3R

Course of the Short Note sn20260416-13r

Short Note submitted on: February 09, 2026

Editorial response: March 09, 2026

Subject: Short Note 013 - Revision requested after peer review

Dear Dr Pauline Henrot and co-authors,

Following peer review, your Short Note entitled "Optimization of human primary myoblasts culture from cryopreserved explants, adhesion, and differentiation on micropatterned soft substrates" requires revision.

Please submit a substantially revised version of your manuscript, together with updated point-by-point responses to the reviewers and to the editor in the review-tracking space. This will initiate a second round of review with the same reviewers.

When responding in the interface, please explain clearly how each comment has been addressed in the revised Short Note. To facilitate evaluation, please indicate for each change either the relevant line number or the beginning of the modified text passage (or the corresponding figure/table legend, where applicable). If you disagree with a comment, please provide a brief justification.

A. Mandatory formatting / editorial corrections

In addition to the reviewers' comments below, I would like to draw your attention to the following editorial points, which should be addressed in the revised version of the manuscript.

1. Cover page
=> The corresponding author should be clearly indicated on the cover page, together with the corresponding email address.
=> Abstract: please spell out PDMS in full at first mention in the abstract.
=> Keywords: from an indexing perspective, the current keywords might be strengthened by using terms such as skeletal muscle - cell adhesion - myogenesis, which would appear both relevant and effective for this Short Note.

If the title, abstract, or keywords are modified in the revised manuscript, please also update the corresponding fields in the submission form to ensure full consistency throughout the submission.

2. Required manuscript sections
Please ensure that all sections requested in the FC3R Short Notes format are present and clearly identifiable in the manuscript. In particular, the revised version should include:
=> an Authors contributions section;
=> a distinct Ethics approval / regulatory information section, where appropriate;
=> explicit funding information in the Acknowledgments section, where applicable.

If funding information is added or revised, please also update the corresponding fields in the submission form where relevant. Research funding agencies such as the ANR, as well as other funders and public research institutions, are paying increasing attention to this type of open science output, including publications disseminated outside traditional Web of Science-indexed journals.

3. Reference citation style
Please harmonize the in-text citation style throughout the manuscript using numbered references in square brackets, in accordance with the Short Notes format.

4. Donor / sample reporting
Please check donor reporting carefully for clarity and consistency. In the current version, one donor code appears to be duplicated and should be verified. Please also ensure that donor characteristics, including sex distribution where relevant, are reported as clearly as possible.

5. Language and consistency revision
As FC3R Short Notes are not copyedited after acceptance, the manuscript would benefit from a careful final English and consistency revision. A few wording or typographical issues appear to remain in the current version and may merit verification in the revised manuscript. Some formulations might possibly be improved, provided

that the authors confirm that the revised wording remains accurate and does not alter the intended meaning :
- "Here, we seeked to optimize..." -> "Here, we sought to optimize..."
- "two explant-based culture" -> "two explant-based culture methods" or "two explant-based cultures"
- "leaded to" -> "led to"
- "favorized" -> "favored" / "promoted"
- Donor code duplicated: "F67, F67"
- "which correspond to the length" -> "which corresponds to the length"
- "coverslip-based explant method was evaluated as similar the Matrigel-based method" -> "was found to be comparable to the Matrigel-based method" ou "was evaluated as being similar to..."
- "making this platform as a promising tool" -> "making this platform a promising tool"
- "nurses form the orthopeadic surgery department" -> "nurses from the orthopaedic surgery department"
- "obtention of human muscle tissue samples" -> "collection of human muscle tissue samples" ou "procurement of..."
- "allowing to retrieve" -> "allowing the collection of" ou "which allowed us to obtain".

6. Visual abstract
Finally, your Short Note would lend itself particularly well to a visual abstract. This could be very useful for communication and promotion, and would likely increase the visibility of your Short Note if accepted. I am attaching three examples for illustration, and I would be very happy if you would consider this possibility.

B. Scientific / methodological revisions requested by reviewers

Please address all reviewer comments carefully as mentionned and revise the manuscript accordingly.

QUALITY OF WRITING _____

- Reviewer 1 ticked : yes

- Reviewer 2 ticked : yes
Reviewer 2 comment :
The article is clear and well written and the results are not over-interpreted.

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS _____
- Reviewer 1 ticked : yes

- Reviewer 2 ticked : no
Reviewer 2 comment :
I have some remarks/quastions.
For the first figure, the image of the myotubes could be improved in terms of resolution (perhaps this was due to my computer, but I had difficulty seeing the myotubes).
Authors' response:

On which day of differentiation was this image taken?
Authors' response:

If the authors had an image with a differentiation marker for this experiment, it would be better to clearly visualize the myotubes.
Authors' response:

Are the fusion index and the percentage of myogenicity the same in both cases?
Authors' response:

There is no scale bar in Figure 1A (I assume it is the same as in 1B). In the text, it is stated that myoblasts appear earlier but in a non-significant manner: how was this quantified?
Authors' response:

- For Figure 1, I would have added a schematic of the experimental setup above the images and, as mentioned earlier, included markers such as an early marker like PAX7 and a late marker like MF20, for example. I would also have added graphs with the numerical values mentioned in the text, even if they are not statistically significant.
Authors' response:

For Figure 2A, the shape of the cells appears very different on the micropatterns, which supports what you describe in the text. However, you only provide a quantification of adhesion. Did you also quantify cell spreading? That would be a useful addition. And what about the fusion index?
Authors' response:

In Figure 2B, it is the coating with fibronectin as said in the texte, but this should be also specified in the figure legend.Do you never obtain this level of differentiation with laminin? This is why it would be useful to specify the fusion index.
Authors' response:

In the discussion, you mention integrins--did you test their expression depending on the coating (in 6-well plates, not on micropatterns)? I understand this is complicated, so it is just a rhetorical question.
Authors' response:

QUALITY OF THE EXPERIMENTAL DESIGN _____

- Reviewer 1 ticked : no
Reviewer 1 comment :
The qualitative observations presented are convincing and well illustrated. However, additional quantitative information would reinforce the methodological comparison and enhance reproducibility.
In particular, the authors may consider clarifying:
- The number of biological and technical replicates performed for each experiment.
Authors' response:
- Whether proliferation rates were formally assessed.
Authors' response:
- Whether fusion efficiency (fusion index) was quantified.
Authors' response:
- The number of fibers analyzed for sarcomere periodicity and whether inter-donor variability was examined.
Authors' response:

- Reviewer 2 ticked : no
Reviewer 2 comment :
For Figure 2, error bars are shown, but do they correspond to the mean across your number of patterns, or did you perform several technical replicates (i.e., repeating the experiment at two different passages)? Error bars and statistical significance are shown, but which statistical test was used?
Authors' response:

As mentioned in the first comment, you state that cells emerge earlier with the coverslip, but without statistical significance. What is the statistical power of your test to determine whether this lack of significance might be due to the small sample size (which is understandable given the difficulty of obtaining biopsies), and therefore to insufficient statistical power?
Authors' response:

Finally, how do you explain that P2 did not respond like the other two patients regarding laminin vs fibronectin? Do you have any idea? This could slightly affect the statistics, because if the patients are considered as n and an overall analysis is performed, the statistical differences between fibronectin and laminin might be less clear. This is why I think that reporting the myogenicity rate or the fusion index for P1/P2/P3 would be important information.
Authors' response:

QUALITY OF THE REPORTING _____

- Reviewer 1 ticked : yes

- Reviewer 2 ticked : yes
Reviewer 2 comment :
Just a small detail: you indicate the concentration of Matrigel, but what volume does this correspond to in a 6-well plate?
Authors' response:

FINAL REVIEWERS DECISIONS _____

- Reviewer 1 final decision : to be discussed
Reviewer 1 final comment :
This short note provides useful technical optimizations for culturing human primary myoblasts on micropatterned soft substrates and contributes to the development of more physiologically relevant in vitro skeletal muscle systems. With minor clarifications and additional quantitative details, this short note would represent a solid and reproducible methodological contribution to the field.
The study provides useful technical insights. The following comments are intended to further strengthen clarity, reproducibility, and interpretation.

Major comments
1- The qualitative observations presented are convincing and well illustrated. However, additional quantitative information would reinforce the methodological comparison and enhance reproducibility.
In particular, the authors may consider clarifying:
- The number of biological and technical replicates performed for each experiment.
Authors' response:
- Whether proliferation rates were formally assessed.
Authors' response:
- Whether fusion efficiency (fusion index) was quantified.
Authors' response:
- The number of fibers analyzed for sarcomere periodicity and whether inter-donor variability was examined.
Authors' response:
Even brief quantitative indications would strengthen the conclusions without substantially expanding the scope of the short note.
Authors' response:

2- Since the comparison between fibronectin and laminin is central to the manuscript, additional methodological details would be helpful for reproducibility:
- Please specify the laminin isoform used.
Authors' response:
- Indicate whether coating densities were normalized between fibronectin and laminin conditions.

Authors' response:
Providing this information would ensure clearer interpretation of the adhesion differences observed.
Authors' response:

3- The experimental design integrates three key parameters: physiological stiffness (12 kPa), rectangular micropatterning, and ECM ligand identity. While the combined approach is appropriate for model development, a brief discussion clarifying the potential relative contribution of stiffness, geometric confinement, and ECM composition would be valuable.
Even if these parameters were not experimentally decoupled, acknowledging this point as a limitation would strengthen the mechanistic interpretation.
Authors' response:

4- The detection of sarcomeric periodicity is an important strength of the study. If available, complementary assessment of late maturation markers (e.g., MYH isoforms) could further support the claim of terminal differentiation. If not within the scope of this short note, a brief mention in the discussion could be considered.
Authors' response:

Minor wording suggestion
The sentence:
"Fibronectin seemed to be the preferred ECM for cellular adhesion."
could be refined for precision as follows:
"Fibronectin appears to provide a more permissive adhesive and mechanotransductive interface for activated human primary myoblasts under micropatterned soft substrate conditions."
This formulation better reflects the biological interpretation of the findings.
Authors' response:

- Reviewer 2 final decision : to be discussed
Reviewer 2 final comment :
In conclusion, there are some modifications that could be made to improve the manuscript, but it is entirely within the scope of a short note. In particular, a few small but important pieces of information are missing in the figure legends, as well as some key data that could potentially explain the differences observed for P2 compared to the others.
Authors' response:

Thank you for your work and for your careful consideration of both the reviewers' and

editorial comments. We appreciate your contribution to the Short Notes initiative and look forward to receiving your revised manuscript.

Best regards,
Marc Le Bert
The Short Notes' editorial board

Author's response: March 31, 2026

A. Mandatory formatting / editorial corrections In addition to the reviewers' comments below, I would like to draw your attention to the following editorial points, which should be addressed in the revised version of the manuscript.

1. Cover page

=> The corresponding author should be clearly indicated on the cover page, together with the corresponding email address. This is now indicated on the cover page.

=> Abstract: please spell out PDMS in full at first mention in the abstract. This has been done, as well as in the first occurrence in the text (p.3).

=> Keywords: from an indexing perspective, the current keywords might be strengthened by using terms such as skeletal muscle - cell adhesion - myogenesis, which would appear both relevant and effective for this Short Note. Following the suggestion, the keywords have been updated as follows: skeletal muscle, cell adhesion, myogenesis. If the title, abstract, or keywords are modified in the revised manuscript, please also update the corresponding fields in the submission form to ensure full consistency throughout the submission.

2. Required manuscript sections

Please ensure that all sections requested in the FC3R Short Notes format are present and clearly identifiable in the manuscript. In particular, the revised version should include:

=> an Authors contributions section; This has now been added p.11 of the revised version.

=> a distinct Ethics approval / regulatory information section, where appropriate; This has now been added p.11-12 of the revised version.

=> explicit funding information in the Acknowledgments section, where applicable. This

has now been added in the Acknowledgements section, p.11 of the revised version. If funding information is added or revised, please also update the corresponding fields in the submission form where relevant. Research funding agencies such as the ANR, as well as other funders and public research institutions, are paying increasing attention to this type of open science output, including publications disseminated outside traditional Web of Science-indexed journals.

3. Reference citation style

Please harmonize the in-text citation style throughout the manuscript using numbered references in square brackets, in accordance with the Short Notes format. This has now been done.

4. Donor / sample reporting

Please check donor reporting carefully for clarity and consistency. In the current version, one donor code appears to be duplicated and should be verified. I confirm that the donor information reported is correct and there was no donor code duplication: the two "F67" correspond to two different female patients of the same age in the cohort. However, while checking the donor information throughout the manuscript, I realized that the donors' informations had been mislabelled in Figure 2A's legend. It initially indicated P1 = F67, P2 = F77 and P3 = M72, but the correct version is P1 = M72, P2 = F77 (correct) and P3 = F71. We apologize for this mistake. This is now corrected in the Figure 2 legend of the revised version.

Please also ensure that donor characteristics, including sex distribution where relevant, are reported as clearly as possible. I confirm that the donor information is now reported everywhere relevant (including in the Figure legends).

5. Language and consistency revision

As FC3R Short Notes are not copyedited after acceptance, the manuscript would benefit from a careful final English and consistency revision. A few wording or typographical issues appear to remain in the current version and may merit verification in the revised manuscript. Some formulations might possibly be improved, provided that the authors confirm that the revised wording remains accurate and does not alter the intended meaning :
- "Here, we seeked to optimize..." -> "Here, we sought to optimize..."
- "two explant-based culture" -> "two explant-based culture methods" or "two explant-based cultures"
- "leaded to" -> "led to"
- "favorized" -> "favored" / "promoted"

Thank you for these suggestions, all the changes have been made.

- Donor code duplicated: "F67, F67" We double-checked the information and the donor code was not duplicated but corresponds to two different women of the same age (please see also above and below).
- "which correspond to the length" -> "which corresponds to the length"
- "coverslip-based explant method was evaluated as similar the Matrigel-based method" -> "was found to be comparable to the Matrigel-based method" ou "was evaluated as being similar to..."
- "making this platform as a promising tool" -> "making this platform a promising tool"
- "nurses form the orthopeadic surgery department" -> "nurses from the orthopaedic surgery department"
- "obtention of human muscle tissue samples" -> "collection of human muscle tissue samples" ou "procurement of..."
- "allowing to retrieve" -> "allowing the collection of" ou "which allowed us to obtain".

All the suggested changes have been made in the revised version. We also carefully checked the remaining document for typos or wording errors and corrected them where necessary, with the help of a collaborator (English level C2), who is now cited in the acknowledgements.

6. Visual abstract

Finally, your Short Note would lend itself particularly well to a visual abstract. This could be very useful for communication and promotion, and would likely increase the visibility of your Short Note if accepted. I am attaching three examples for illustration, and I would be very happy if you would consider this possibility. We propose the following Graphical Abstract (appended to the submission).

B. Scientific / methodological revisions requested by reviewers

Please address all reviewer comments carefully as mentionned and revise the manuscript accordingly.

QUALITY OF WRITING

- Reviewer 1 ticked : yes
- Reviewer 2 ticked : yes

Reviewer 2 comment : The article is clear and well written and the results are not over-interpreted.

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS
- Reviewer 1 ticked : yes
- Reviewer 2 ticked : no

Reviewer 2 comment : I have some remarks/quastions. For the first figure, the image of

the myotubes could be improved in terms of resolution (perhaps this was due to my computer, but I had difficulty seeing the myotubes).

Authors' response:
We agree with the Reviewer that the resolution does not particularly allow to see myotubes (we also attach a bigger version of the same picture in the point-by-point response submitted as an appendix). Also, as asked below, this picture was taken at day 5 of differentiation, therefore relatively early. In line with the other comments below, we have decided to replace the picture by an immunostaining of late differentiation markers (alpha-actinin, Myosin Heavy Chain) in order to better show the ability of muscle cells to differentiate into myotubes (please see answers below as well as the new version of Figure 1).

On which day of differentiation was this image taken?
Authors' response:
This image was taken at day 5 of differentiation.

If the authors had an image with a differentiation marker for this experiment, it would be better to clearly visualize the myotubes.
Authors' response:
We indeed performed immunostaining on fixated myotubes for the same patient (but sadly, from the Matrigel technique only, as we could not find fixated slides from myotubes obtained with the coverslip technique). We attach the result of the immunostaining in the point-by-point response submitted as an appendix. Alpha-actinin is represented in cyan, Myosin Heavy Chain in magenta and nuclei (stained with DAPI) in white (scale bar: 50 µm). The staining allows to evidence differentiated myotubes (although not over the whole image). Alpha-actinin also allows the visualization of sarcomeres as highlighted in the close-up picture below (scale bar: 20 µm).

Following the Reviewer's recommendation, we have updated Figure 1 with the staining in order to better evidence the ability of cultured myotubes to differentiate.

Are the fusion index and the percentage of myogenicity the same in both cases?
Authors' response:
Sadly, we did not perform immunostainings on fixated myotubes from both techniques within the same experiment. Therefore, we are unable to formally compare the amount of differentiation between both techniques. There is no scale bar in Figure 1A (I assume it is the same as in 1B).

In the text, it is stated that myoblasts appear earlier but in a non-significant manner: how was this quantified?
Authors' response:
The scale bar (which was indeed the same) is now added, thank you for pointing this out. The quantification of myoblasts appearance was performed visually, with careful visualization of each explant under the microscope every day. Following the suggestion

below (please see the next question), we added a graph representing the days of first myoblasts appearance for each of the six donors tested. We realized at this occasion that we had used the wrong statistical test to analyze the data, which should have been analysed using a paired non-parametric t-test instead of an unpaired non-parametric test as we first did. With this new comparison, the first emerging cells were seen earlier in the coverslip technique in a significant manner. However, there was still no significant difference in the number of days before the first passage in both techniques.
- For Figure 1, I would have added a schematic of the experimental setup above the images and, as mentioned earlier, included markers such as an early marker like PAX7 and a late marker like MF20, for example.

I would also have added graphs with the numerical values mentioned in the text, even if they are not statistically significant.
Authors' response:
Following the Reviewer's recommendation, we added the experimental setup in the Figure 1A panel (which can also be implemented in the Methods in case the figure is too big). We also added graphs with the numerical values for each of the six donors for first emerging cells and first passage. We realized at this occasion that we had used the wrong statistical test to analyze the data, which should have been analysed using a paired non-parametric t-test (Wilcoxon's test) instead of an unpaired non-parametric test as we first did. With this new comparison, the first emerging cells were seen earlier in the coverslip technique in a significant manner. However, there was still no significant difference in the number of days before the first passage in both techniques. The text has been updated with these new informations (please see p.4, lines 96-100). We also added the immunostaining with late differentiation markers (alpha-actinin and Myosin Heavy Chain, clone MF-20 indeed) as discussed above. Sadly, we did not perform any immunostaining with Pax7.
For Figure 2A, the shape of the cells appears very different on the micropatterns, which supports what you describe in the text. However, you only provide a quantification of adhesion.

Did you also quantify cell spreading? That would be a useful addition. And what about the fusion index?
Authors' response:
We thank the Reviewer for the opportunity to clarify. Here, we quantified what we initially described as the percentage of adhered cells (visual quantification of cell surface over the total pattern surface), which in this case also corresponds to the level of spreading, as the images have been taken 24 hours after seeding. Sadly, we did not record images of cells which were adhered but not spread (which would correspond to a few hours after seeding). We updated the text and figure legend mentioning the quantification of the level of cell spreading. Concerning the fusion index: for the experiment presented in Figure 2A, the cells have been fixated and imaged 24 hours after seeding, which seems too early to assess the fusion index. Our primary aim with this experiment was to determine which of the two ECM molecules would be more suitable for cell adhesion and spreading, but not for cell differentiation. To make it clear

for readers, we have updated the text with this information (please see p.5-6 lines 131-136), as well as discussed this point in the Discussion section (please see p.7 lines 184-192), including the available literature data comparing the effects of laminin and fibronectin with regards to differentiation. We also removed the term "differentiation" from the title in order not to mislead the readers. In Figure 2B, it is the coating with fibronectin as said in the texte, but this should be also specified in the figure legend.

Do you never obtain this level of differentiation with laminin? This is why it would be useful to specify the fusion index.
Authors' response:
We thank the Reviewer for pointing this out and added the information of the fibronectin coating in the figure legend. As precised above, sadly, we did not perform such immunostaining on laminin-coated patterns at this timepoint (7 days of differentiation), which prevents us from comparing the fusion index in both cases. As discussed above, our aim was primarily to assess the difference in ability to adhere and spread and not to differentiate. We have discussed this point with regards to the available literature in the Discussion section (please see p.7).

In the discussion, you mention integrins--did you test their expression depending on the coating (in 6-well plates, not on micropatterns)? I understand this is complicated, so it is just a rhetorical question.
Authors' response:
We thank the Reviewer for this relevant point, which would indeed support our hypothesis. We have not performed such experiment, but it would definitely be of interest for further studies. We added this suggestion in the discussion (please see p.7 lines 175-176).

QUALITY OF THE EXPERIMENTAL DESIGN

- Reviewer 1 ticked : no
Reviewer 1 comment : The qualitative observations presented are convincing and well illustrated. However, additional quantitative information would reinforce the methodological comparison and enhance reproducibility. In particular, the authors may consider clarifying:
- The number of biological and technical replicates performed for each experiment.
Authors' response:
We thank the Reviewer for raising this point. For each experiment, we clarified in the Figure legend the number of biological and technical replicates where necessary or missing (please see the legends of Figure 1 and 2 in the revised version).
- Whether proliferation rates were formally assessed.
Authors' response:
Sadly, we did not formally assess proliferation rates from the two methods, which we had acknowledged in the initially submitted version (please see p.4 line 111).
- Whether fusion efficiency (fusion index) was quantified. As discussed above, sadly,

we did not fixate myotubes obtained from both techniques within the same experiment and for the same donor, and therefore are unable to perform a comparative analysis of the fusion index. To make it clear for readers, we have now acknowledged this in the revised version (please see p.4 lines 102-103).
Authors' response:
- The number of fibers analyzed for sarcomere periodicity and whether inter-donor variability was examined.
Authors' response:
Only the displayed fiber in Figure 2B was analysed for sarcomere pediodicity in the initially submitted version. Following the Reviewer's suggestion for quantitative indications, we performed additional imaging of the fixated and stained slides in order to image more myotubes. The results of these new quantifications (5 myotubes analysed) are presented in the point-by-point response submitted as an appendix, with the red line indicating the mean and the black lines the standard deviation. However, pooling these results led to more variability and the graph was less indicative of the level of differentiation, possibly due to the need for a higher resolution (which was not possible for our microscope (epifluorescence)). Therefore, for illustrative purposes, we decided to display the initial graph. To make it clear for readers, we added in the legend of Figure 2B "Quantification was performed on 5 different zones from differentiated myofibres on images taken at x40 magnification, and the graph corresponding to the most differentiated zone is displayed." Concerning inter-donor variability, only one donor was used for the immunostaining experiment, therefore inter-donor variability was not assessed. This information has been added to the figure legend and text (please see p.6 line 139 + new version of Figure 2B/C legend).

- Reviewer 2 ticked : no
Reviewer 2 comment : For Figure 2, error bars are shown, but do they correspond to the mean across your number of patterns, or did you perform several technical replicates (i.e., repeating the experiment at two different passages)? Error bars and statistical significance are shown, but which statistical test was used?
Authors' response: We thank the Reviewer for the opportunity to clarify and complete our description of the experiment. Micropatterns fabricating and sterilization was quite challenging and there was a limited number of experiments that we could perform due to a limited number of available micropatterns surfaces. We chose to assess biological variability, using three different donors, instead of technical variability, because we were more interested in inter-donor variability in this case. We added this information in the Figure legend. The error bars here correspond to the mean across the number of patterns indeed. The same graph but displaying individuals dots (1 dot = 1 pattern) is shown in the point-by-point response submitted as an appendix for your convenience. For the Figure, we chose a histogram representation because we thought it would be more visual in evidencing the difference. The statistical test used was a 2way Anova with Šídák's multiple comparisons test. This information as well as the corresponding p-values have also been added in the text and the Figure legend (please see p.6, line 134, as well as new version of Figure 2A legend). As mentioned in the first comment, you state that cells emerge earlier with the coverslip, but without

statistical significance.

What is the statistical power of your test to determine whether this lack of significance might be due to the small sample size (which is understandable given the difficulty of obtaining biopsies), and therefore to insufficient statistical power?
Athors' response: We also thank the Reviewer for the opportunity to clarify. This point had also been raised by the other Reviewer. We realized at this occasion that we had used the wrong statistical test to analyze the data, which should have been analysed using a paired non-parametric t-test (with paired data per donor) instead of an unpaired non-parametric test as we first did. With this new comparison, the first emerging cells were seen earlier in the coverslip technique in a significant manner. The abstract, text and the Figure 1C have all been updated with these new informations (please see p.4, line 96-100, and new version of Figure 1C legend). Finally, how do you explain that P2 did not respond like the other two patients regarding laminin vs fibronectin? Do you have any idea? This could slightly affect the statistics, because if the patients are considered as n and an overall analysis is performed, the statistical differences between fibronectin and laminin might be less clear. This is why I think that reporting the myogenicity rate or the fusion index for P1/P2/P3 would be important information.
Authors' response: We thank the Reviewer for raising this important point. As for the statistical test, we have performed a 2way ANOVA with Šídák's multiple comparisons test for this experiment, allowing us to conclude that the statistical difference remains significant overall despite the lack of difference for the patient P2. This information as well as the corresponding p-values have also been added in the Figure legend and in the text (please see p.6, line 134, as well as new version of Figure legend 2A). As for the reason why P2 did not respond the same way as the other patients, we cannot give any definite explanation, but we do believe inter-donor variability plays a strong role. From the available clinical data: P2 was a 77 yo female with no known muscle disease and normal BMI (BMI 19.5); P1 was a 72 yo male with no known muscle disease and overweight (BMI 35); (we had initially provided partially wrong patient information in the firstly submitted version, for which we apologize, this is now corrected); P3 was a 71 yo female with no known muscle disease and slightly overweight (BMI 27.7). Therefore, one clinical characteristic that differs between P2 and P1-P3 is the weight (normal vs high), however this is too preliminary to allow us to conclude that this is the cause of the difference. We could speculate that as the ECM remodelling is likely to be different in obese versus non-obese skeletal muscle tissue, it might lead to different integrins expression, but we cannot assume that such differential expression would remain in vitro. Due to space constraints, we propose not to include these specific elements in the Discussion, however we added a sentence in the Discussion to stress out the likely role of inter-donor variability in this discrepancy as well as the necessity to expand the number of donors in order to be able to draw definite conclusions (please see p.7, lines 176-179).

QUALITY OF THE REPORTING

- Reviewer 1 ticked : yes

- Reviewer 2 ticked : yes
Reviewer 2 comment :
Just a small detail: you indicate the concentration of Matrigel, but what volume does this correspond to in a 6-well plate?
Authors' response:
We thank the Reviewer for the opportunity to clarify; the Matrigel solution was applied as a drop over each explant, with a p1000 pipette (so approximatively 150-200 µL for each explant), rather than covering the whole well. This is now clarified in the Methods section (please see p.13, lines 397-399).

FINAL REVIEWERS DECISIONS

Reviewer 1 final decision : to be discussed
Reviewer 1 final comment :
This short note provides useful technical optimizations for culturing human primary myoblasts on micropatterned soft substrates and contributes to the development of more physiologically relevant in vitro skeletal muscle systems. With minor clarifications and additional quantitative details, this short note would represent a solid and reproducible methodological contribution to the field. The study provides useful technical insights. The following comments are intended to further strengthen clarity, reproducibility, and interpretation. Major comments 1- The qualitative observations presented are convincing and well illustrated. However, additional quantitative information would reinforce the methodological comparison and enhance reproducibility. In particular, the authors may consider clarifying:

- The number of biological and technical replicates performed for each experiment.
Authors' response:
We have now carefully checked that the information was present throughout the whole document (please see the corresponding point above).

- Whether proliferation rates were formally assessed.
Authors' response:
As precised in the initially submitted version (please see p.4 line 111), sadly, at the time we had not formally assessed proliferation rates between the two techniques.

- Whether fusion efficiency (fusion index) was quantified.
Authors' response:
As discussed above, our aim was to compare the effect of the ECM molecule (laminin or fibronectin) on cellular adhesion and spreading and not on differentiation into myotubes; therefore, we did not assess fusion index (and sadly, the fixation timepoints were too early to assess this). Please also see the answer to this question above. We have added the potential effect on differentiation in the new version of the Discussion.

- The number of fibers analyzed for sarcomere periodicity and whether inter-donor variability was examined.

Authors' response:
We thank the Reviewer for raising this point. Only one donor was used for the experiment shown in Figure 2B (which is a different experiment than in Figure 2A), with 3 technical replicates. In the first submitted version, only one zone had been analysed for sarcomere periodicity.

As discussed above, and following the Reviewer's suggestion for quantitative indications, we performed additional imaging of the fixated and stained slides in order to image more myotubes. The results of these new quantifications (5 myotubes analysed) are presented in the point-by-point response submitted as an appendix, with the red line indicating the mean and the black lines the standard deviation. However, pooling these results led to more variability and the graph was less indicative of the level of differentiation, possibly due to the need for a higher resolution (which was not possible for our microscope (epifluorescence)). Therefore, for illustrative purposes, we decided to display the initial graph. To make it clear for readers, we added in the legend "Quantification was performed on 5 different zones from differentiated myofibres on images taken at x40 magnification, and the graph corresponding to the most differentiated zone is displayed." Even brief quantitative indications would strengthen the conclusions without substantially expanding the scope of the short note.
Authors' response:
We agree and thank the Reviewer for this opportunity.

2- Since the comparison between fibronectin and laminin is central to the manuscript, additional methodological details would be helpful for reproducibility:

- Please specify the laminin isoform used.
Authors' response:
We enquired the supplier (Roche) about this and the answer was "Laminin-1: alpha1beta1gamma1 (Laminin-111)". This information has now been added in the Methods (please see p.14 line 439). It is indeed of importance, as we have found a study comparing the effects of different laminin isoforms on myoblasts differentiation into myotubes and evidencing a better differentiation with the isoform 521. We have updated the Discussion with this information (please see p.7 lines 188-192).

- Indicate whether coating densities were normalized between fibronectin and laminin conditions. Authors' response: The same concentration of fibronectin and laminin (50 µg/mL) was used to prepare the coating solution as precised in the Methods in the initially submitted version (please see p.14 lines 439-440).

Providing this information would ensure clearer interpretation of the adhesion differences observed.
Authors' response:
We completely agree and had provided this information indeed.

3- The experimental design integrates three key parameters: physiological stiffness (12

kPa), rectangular micropatterning, and ECM ligand identity. While the combined approach is appropriate for model development, a brief discussion clarifying the potential relative contribution of stiffness, geometric confinement, and ECM composition would be valuable. Even if these parameters were not experimentally decoupled, acknowledging this point as a limitation would strengthen the mechanistic interpretation.
Authors' response:
We completely agree with the Reviewer about the independent importance of each of these three points. The 12 kPa stiffness had been selected from the literature, having been measured as the physiological muscle tissue stiffness and having been shown as the optimal value for muscle cells differentiation (PMID: 15364962). The rectangular shape has also been chosen as based on examples from the literature (PMID: 26983843) as well as the shape of an elongated myofibre in vitro. These elements have been added to the new version of the Discussion.

Finally, the influence of the chosen ECM ligand has been discussed in the new version of the Discussion following the questions raised above. Concerning the relative contribution of each of these parameters, it is difficult to speculate but we would propose that the combination of physiological stiffness and the most adapted ECM ligand would definitively be needed for differentiation; the geometric confinement would be more useful for myotube alignment. We have also added this proposition in the Discussion. Please find the new paragraph p.8 lines 201-209.

4- The detection of sarcomeric periodicity is an important strength of the study. If available, complementary assessment of late maturation markers (e.g., MYH isoforms) could further support the claim of terminal differentiation. If not within the scope of this short note, a brief mention in the discussion could be considered.
Authors' response:
We thank the Reviewer for this suggestion, however, we have only performed Myosin Heavy Chain staining for this experiment and have not stained for the different Myosin Chain isoforms (due to the limited number of channels available for the staining: 2, this would have prevented us from using alpha-actinin and therefore would have impaired the sarcomere periodicity analysis). Following the Reviewer's suggestion, we have now mentioned this limitation in the Discussion (please see p.7-8, lines 197-200).

Minor wording suggestion The sentence: "Fibronectin seemed to be the preferred ECM for cellular adhesion." could be refined for precision as follows: "Fibronectin appears to provide a more permissive adhesive and mechanotransductive interface for activated human primary myoblasts under micropatterned soft substrate conditions." This formulation better reflects the biological interpretation of the findings.
Authors' response:
We thank the Reviewer for this suggestion and have modified the corresponding sentence in the Discussion (please see p.7 lines 169-171).

- Reviewer 2 final decision : to be discussed

Reviewer 2 final comment : In conclusion, there are some modifications that could be made to improve the manuscript, but it is entirely within the scope of a short note. In particular, a few small but important pieces of information are missing in the figure legends, as well as some key data that could potentially explain the differences observed for P2 compared to the others.
Authors' response:
We thank the Reviewer for her/his careful reading of our manuscript and opportunity to improve the precision of the reported data.

Thank you for your work and for your careful consideration of both the reviewers' and editorial comments.

We appreciate your contribution to the Short Notes initiative and look forward to receiving your revised manuscript.

We are thankful to the Reviewers and Editor for the opportunity to improve our manuscript and precise our conclusions.

Due to the requested changes, the word count is now above the limit (2322 words), for which we apologize.

We look forward to your response to our revised version.

Best regards, Marc Le Bert The Short Notes' editorial board

Revised version of the Short Note submitted on: March 31, 2026

Final editorial response: April 16, 2026

Dear Dr Pauline Henrot and co-authors,

I'm pleased to inform you that your Short Note 13, entitled "Optimization of human primary myoblasts culture from cryopreserved explants and adhesion on micropatterned soft substrates", has been accepted for publication.

Congratulations and thank you for carefully addressing the reviewers' and editorial requests, and for your commitment to sharing previously unpublished results. This contributes to greater clarity, accessibility, and transparency, thereby strengthening the robustness of science.

Could you send me the final PDF and Word versios for post-editing? Your Short Note will first be deposited in HAL and assigned a DOI. It will then also be made available on the Short Notes platform, together with the peer-review document annexed to the publication. You will be notified at the end of this process and provided with the corresponding links.

Please also remember to apply to the OPAL Short Notes Challenge to claim the prize offered by the OPAL association. Being selected for this prize may also provide an opportunity for your Short Note to be highlighted during the online FC3R 3Rs Day event on 2 July 2026.

With best regards,
Marc Le Bert
Short Notes Editorial Team

QUALITY OF WRITING _____
Reviewer 1 ticked : yes
Reviewer 2 ticked : yes

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS _____
Reviewer 1 ticked : yes
Reviewer 2 ticked : yes

QUALITY OF THE EXPERIMENTAL DESIGN _____
Reviewer 1 ticked : yes
Reviewer 2 ticked : yes

QUALITY OF THE REPORTING _____
Reviewer 1 ticked : yes
Reviewer 2 ticked : yes

FINAL REVIEWERS DECISIONS _____
Reviewer 1 final decision : yes
Reviewer 2 final decision : yes

Reviewer 1 final comment :
The authors have carefully addressed all the comments raised during the review process. Their revisions have significantly improved the clarity and overall quality of the short note. I therefore support its publication in its current form.

Reviewer 2 final comment :
The short note has been improved since its initial version: the points raised have been revised and, where necessary, discussed. I therefore accept it in its current form. This type of methodological contribution will provide useful information to the community and help save time for those who may need to develop a similar protocol.



Final validation and publication: April 16, 2026