nature portfolio

Peer Review File

Targeting Bradykinin Signaling Pathway: PLGA/BK Microspheres as a Therapeutic Strategy for Delaying Intervertebral Disc Degeneration

Corresponding Author: Professor Xuewen Kang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
- Method details are lacking; please specify, for example, the dilution factor of the antibody in the western blot.
- Avoid excessive use of abbreviations such as IHC and WB in section titles; these require revision.
- The fonts in figures and graphs are too small to read; please enlarge these throughout the paper.
- Clarity of Fig. 1 workflow may be improved.
- Figure 2-4, 8, 9 legends, please indicate number of animals and statistical method
- Fig 4 g, i, 1) seems like the backgrounds of images are high and both channel images lack of resolution. Cell boundaries are blurred and hard to distinguish positive signal loation, esp in MMP9. Have IgG control of Immunofluorescence staining being performed? Also for aggrecan and MMP9, they should be abundent of both protein in extracellular matrix, in addition to intracellular, which may be evaluated to better indicate the change.
- Fig 9, 1) resolution of histology images are too low to review. 2) Safranin-O staining: histology score shall be performed as it provides some semi-quantitatvie measure to assess the degenerative or restorative effect. 3) There seemed to be many big gap between disc AF and growthplate in b images. Are these artifacts or loss of cellularity druing the process? 4) Please add graphic labels like arrows to indicate any significant change that reader should pay attetion. Overall, low quality images made review difficult.

Reviewer #2

(Remarks to the Author)
In this paper, the authors have identified BK as a molecular marker of IVDD and then developed a sustained release formulation of BK using PLGA microspheres. The study involved a thorough investigation of the combined effect of the BK/PLGA group on delaying the progression of IVDD in rat model. The following suggestions may improve the quality of the manuscript before publication in Communications Biology.
1. The title of the paper needs to be modified as it only focuses on the sustained release formulation, excluding the discovery of BK as a therapeutic target for treating IVDD.
2. The authors have denoted BK as a biomaterial in the introduction section. It would be better to term it as a biomolecule or more specifically as a peptide.
3. The authors need to explain the detailed process for measuring the quantity of encapsulated drug within the microsphere. For example, the reason for introducing ultrapure water and centrifugation is not clear.
4. The fonts of Fig. 4 and Fig. 8 are not clearly visible. Also, statistical analysis is missing in some of the graphs in Fig. 4. Are the differences not significant for those graphs?
5. The introduction of section 3.5.1 is abrupt and needs modification.
6. It would be better if the nomenclature of A, B, C, D is mentioned at the bottom of Table 4 and Table 5 for helping readers in correlating the effects.
7. In Fig. 6b (1000X), the size of the particles appears to be increasing after reducing the PLGA content from 100 mg to 50 mg. This is contradictive to the DLS measurement and needs to be explained properly.
8. The particle size is not demonstrated by Table 6, but mentioned wrongly in the caption and corresponding text in the explanation. Also, the effect of particle size reduction should be correlated with the drug loading rate.
9. The drug release data should be explained in more detail. The reason for pH responsive release of BK from PLGA

microspheres is not clear.
10. From the in vivo data, it appears that the effect of BK/PLGA group is predominantly visible till 1 month and disappears after 2 months. Since the drug release data (Fig. 7e) shows almost 80% release after 1 month, the remaining amount of drug might not be enough to cause significant improvement in the disc health after 2 months. A clear explanation along this aspect may be added in the discussion section.

Reviewer #3

(Remarks to the Author)
Marks to the Author:
In this study, researchers combined clinical data and transcriptome sequencing to identify the involvement of BK in the cellular aging process, and prepared PLGA/BK microspheres through process optimization to save nucleus pulposus cell aging and improve extracellular aging by regulating the B2R and PI3K pathways. The synthesis and metabolism of substrates provide a feasible strategy for delaying IVDD. The author's data supports the proposed viewpoint, but it is recommended to make revisions to the article before accepting the manuscript:
1.There are too many numbers and text in the manuscript images to display clearly. The author should reorganize key data, improve image quality, or display some images that are not the main points of supplementary materials.
2.In our common understanding, BK is a pro-inflammatory mediator. Will inflammation in the intervertebral disc increase during BK delivery to IVDD? The author should add experimental explanations.
3.Nanomicrospheres may have a rapid loss probability when injected into intervertebral discs in situ.Suggest the author to add data on the retention time of PLAG/BK nanospheres in vivo.
4.The discussion section of the manuscript should be revised to enhance the logical coherence of the viewpoints, rather than piling up result data.
The completeness of the article is good, but the quality of the images and the writing of the manuscript should be strengthened.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The reviewer appreciates the thorough revisions from the authors. Only one issue was discovered: the scale bar for the second row of Fig 5A is missing.

Reviewer #2

(Remarks to the Author)
I appreciate the authors for their sincere efforts in improving the quality of the manuscript. The current version of the manuscript may be accepted for publication.

Reviewer #3

(Remarks to the Author)
Thank you to the author for their positive response to my review comments. But before the manuscript is accepted, the author still needs to make modifications to the following issues. Otherwise, I do not recommend accepting the manuscript.
1.There is a lot of debate about BK in the study of intervertebral disc apoptosis, and the author has achieved different results through previous data research. Discussions can be appropriately expanded.
2.The author verified this method through human body data, and it is very effective. However, the positive rate of BK in fig1A does not seem to be significant.
3.The author can refer to other articles in the journal to improve the presentation of the entire article, and the methods and conclusions should be clearly distinguished.
4.The author should enhance the discussion mode rather than repeating the results.
5.Simplify the conclusion.
6.I did not see the expression of the core data BK in degenerative mice. Can I supplement this key data to make the entire article more convincing.
7.The full text images should be significantly modified to better showcase the core content of the manuscript.

Reviewer #4

(Remarks to the Author)
The study by Qiu et al. examines the effects and underlying mechanisms of poly(lactic-co-glycolic acid)/Bradykinin (PLGA/BK) microspheres on intervertebral disc degeneration (IVDD). The manuscript is well-written but lacks important details necessary to understand the rigor of the work.

Methods:
1. The term "whole-gene transcriptome" is confusing. Are the authors referring to targeted transcriptome sequencing or bulk

RNAseq sequencing/whole transcriptome sequencing? Please clarify.
2. The methods section lacks a lot of details on the RNA extraction kit, library QC, sequencing technology, and bioinformatics analysis. Specifically:
a. Specify the RNA extraction kit used.
b. Detail any library QC undertaken.
c. Identify the high-throughput sequencing technology and the specific sequencer used.
d. Provide comprehensive information on the bioinformatics steps taken, including tools and their versions, the reference genome used, and any specific QC applied.
e. Explain how the chosen pipeline is suitable for studying cell aging and extracellular matrix metabolism.
f. Are raw RNAseq data being deposited in repositories like GEO?
g. Is the code/pipeline used going to be available?
h. A supplementary figure detailing the bioinformatics steps use might be helpful

Results section:
3. In the results section, they state "analysis identified 22 genes with marked differences ($P < 0.05$)" but do not specify if this is an unadjusted or adjusted p-value. Clarify whether the reported P-value ($P < 0.05$) is adjusted or unadjusted. This distinction is crucial for interpreting the statistical significance of your findings.
4. The authors mentioned "Among these genes, 12 were notably upregulated (Fig. 3D), while 10 exhibited reduced expression (Fig 3E)" but do not specify in which group. Specify in which group the 12 upregulated and 10 downregulated genes were identified.

Figures:
5. In the heatmap (Fig 3C), how do the authors justify that the B2R samples do not show the same expression for genes they claim to be upregulated/downregulated? How have they accounted for sample variability and replicates in their analyses?
6. Ensure the legends in Figure 3 are not hidden. The font also seems small making it difficult to read.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
The author has successfully completed the revision of the manuscript. I have no further comments.

Reviewer #4

(Remarks to the Author)
I was pleased to see that the authors were able to address the clarity and description of the methodology in the paper. For the integrity of sharing and reproducible science, authors should deposit RNAseq data in repositories.

Authors' response to reviewers' comments

Manuscript Number: COMMSBIO-23-4375-A

PLGA/BK Microspheres as a Therapeutic Strategy for Delaying Intervertebral Disc Degeneration

We are grateful to the editor and reviewers for their helpful comments. The manuscript has been revised according to the reviewers' comments and point-by-point response has been made as follows,

Reviewers' comments:

Reviewer #1:

1. Method details are lacking; please specify, for example, the dilution factor of the antibody in the western blot.

Response: Thank you very much for your valuable suggestions. Based on a detailed analysis of our experimental data, we have thoroughly updated and supplemented the details of specific experimental methods in our paper, particularly regarding antibody dilution ratios, among others. We believe these adjustments will make our research findings more accurate and reliable.

2. Avoid excessive use of abbreviations such as IHC and WB in section titles; these require revision.

Response: Thank you for your valuable feedback. Regarding the issue of excessive use of abbreviations in the title, as you mentioned, we have made revisions. Now, all technical terms, including Immunohistochemistry (IHC) and Western Blotting (WB), have been changed to their full descriptive forms.

3. The fonts in figures and graphs are too small to read; please enlarge these throughout the paper.

Response: Thank you for your valuable advice. Following your guidance, we have adjusted the font size in the figures to ensure clarity and facilitate accurate identification, which is crucial for the precise and comprehensive communication of our research findings.

4. Clarity of Fig. 1 workflow may be improved.

Response: Your suggestions are incredibly valuable to us, and we have taken them very seriously. In response, we have comprehensively redesigned the font size, layout, and content of our images to ensure they meet the requirements for clear expression of our research findings

5. Figure 2-4, 8, 9 legends, please indicate number of animals and statistical method.

Response: Thank you very much for your attention to the details of our work. In accordance with your suggestion, we have now clearly indicated the number of animals and the statistical methods used in the legends of Figures 2-4, 8, and 9. This

enhancement will provide readers with a clearer and more detailed context of experimental design and analysis of results, ensuring transparency and reproducibility of our research.

6. Fig 4 g, i, 1) seems like the backgrounds of images are high and both channel images lack of resolution. Cell boundaries are blurred and hard to distinguish positive signal loation, esp in MMP9. Have IgG control of Immunofluorescence staining being performed? Also for aggrecan and MMP9, they should be abundent of both protein in extracellular matrix, in addition to intracellular, which may be evaluated to better indicate the change.
Response: Thank you very much for your detailed observations and valuable suggestions. Regarding the issues you raised about high background, insufficient resolution, and difficulty distinguishing positive signals in Figures 4g, i, and l, we have taken the following improvement measures:

We have re-uploaded high-definition fluorescence original images. This step has significantly improved the image quality, helping us to more clearly distinguish the position of positive signals.

Regarding your comments on the abundance of proteoglycans and MMP9 not only intracellularly but also in the extracellular matrix, we have re-evaluated and supplemented our experimental data. We found that these proteins are indeed difficult to observe in the extracellular matrix, possibly due to the use of cell smears and multiple washes before and after fixation. We attempted to reduce the number of washes; however, this severely affected the staining quality, rendering the results unusable. Therefore, after weighing the pros and cons, we chose to continue with multiple washes.

We sincerely appreciate your valuable suggestion regarding IgG control. After reviewing relevant literature and consulting with the technical support from our antibody supplier, we have confirmed the specificity of the antibodies used. Accordingly, we have re-uploaded the images and statistical data of fluorescence intensity to more robustly support our conclusions. We sincerely apologize for any technical shortcomings and commit to further optimizing our staining process in future experiments to minimize background interference.

With these improvements, we hope to present our experimental results more accurately. Thank you once again for your guidance, which is crucial for improving the quality of our research.

7. Fig 9, 1) resolution of histology images are too low to review. 2) Safranin-O staining: histology score shall be performed as it provides some semi-quantitatvie measure to assess the degenerative or restorative effect. 3) There seemed to be many big gap between disc AF and growthplate in b images. Are these artifacts or loss of cellularity druing the process? 4) Please add graphic labels like arrows to indicate any significant change that reader should pay attetion. Overall, low quality images made review difficult.

Response: Thank you very much for your thorough review and invaluable suggestions. In response to the concerns you raised about Figure 9, we have implemented the following improvements:

1. We have re-uploaded the histology images and ensured their resolution was high enough for detailed examination.

2.For the Safranin-O staining, we have now performed histological scoring. This semi-quantitative method will allow us to more accurately assess the effects of degeneration or recovery.

3.Regarding the gaps observed between the annulus fibrosus and endplates in image b, after enhancing the image resolution, we carefully reviewed the original samples and processing procedures and can now clearly identify these tissue.

4.We have introduced arrows into the figures to clearly indicate significant changes that should capture the reader's attention.

We recognize the paramount importance of image quality in presenting our research findings and have rigorously corrected all the issues raised. We hope these efforts will facilitate a smoother review process. Thank you again for your valuable guidance and patience.

Reviewer #2: In this paper, the authors have identified BK as a molecular marker of IVDD and then developed a sustained release formulation of BK using PLGA microspheres. The study involved a thorough investigation of the combined effect of the BK/PLGA group on delaying the progression of IVDD in rat model. The following suggestions may improve the quality of the manuscript before publication in Communications Biology.

1. The title of the paper needs to be modified as it only focuses on the sustained release formulation, excluding the discovery of BK as a therapeutic target for treating IVDD..
Response: Thank you for your valuable comments, which are crucial for us to accurately convey the core content and highlights of our paper through its title. Following your suggestion, we have revised the title to "Targeting Bradykinin Signaling Pathway: PLGA/BK Microspheres as a Therapeutic Strategy for Delaying Intervertebral Disc Degeneration" to better reflect the dual significant roles of BK as both a therapeutic target and a sustained-release agent.

2. The authors have denoted BK as a biomaterial in the introduction section. It would be better to term it as a biomolecule or more specifically as a peptide.
Response: Thank you for highlighting the inaccuracies in our description of BK in the introduction. Following your recommendation, we have revised the manuscript to more accurately describe BK as a peptide. We agree that this terminology not only more clearly conveys the nature of BK but also enhances the professionalism and precision of our

paper. Thank you once again for your careful review and valuable suggestions.

3.The authors need to explain the detailed process for measuring the quantity of encapsulated drug within the microsphere. For example, the reason for introducing ultrapure water and centrifugation is not clear.

Response: Thank you for your suggestion. We have now added detailed information about the process of measuring the amount of drug encapsulated within the microspheres. Specifically, we have clarified the reasons for using ultrapure water and the centrifugation step: ultrapure water is used to dissolve the drug, ensuring its purity and accurate measurement; centrifugation is utilized to separate the free drug not encapsulated by the microspheres and PVA solution, allowing for precise calculation of the drug quantity within the microspheres. We hope these additions clearly explain the specific steps of the experiment and enhance the transparency and reproducibility of our research. Thank you once again for your valuable feedback.

4.The fonts of Fig. 4 and Fig. 8 are not clearly visible. Also, statistical analysis is missing in some of the graphs in Fig. 4. Are the differences not significant for those graphs?

Response: Thank you very much for pointing out the issues with the font clarity in Figures 4 and 8, and the lack of statistical analysis in certain charts in Figure 4. In response to these issues, we have made the following corrections:

1. We have increased the font size in Figures 4 and 8 to ensure all textual information is clearly readable.

2. Regarding the charts in Figure 4 that were missing statistical analysis, upon further review, we confirmed that these data do not show significant statistical differences; here, we mainly observed trends in variations between groups.

We are fully aware of the importance of clear graphical presentations and complete data analysis in scientific research, and thus we take your suggestions seriously and address them with great care. Thank you once again for your meticulous review and valuable comments.

5.The introduction of section 3.5.1 is abrupt and needs modification.

Response: Thank you for your feedback. We acknowledge that the introduction of Section 3.5.1 was indeed abrupt. To address this, we have thoroughly revised the beginning of 3.5 to ensure a natural transition from the preceding content and clearly state the research objectives and significance of this section. We believe these modifications will make the article's structure more coherent and help guide readers through the core content of this section more effectively. Thank you once again for your valuable suggestions, which have greatly aided us in improving the structure of our manuscript.

6. It would be better if the nomenclature of A, B, C, D is mentioned at the bottom of Table 4 and Table 5 for helping readers in correlating the effects.

Response: Thank you very much for your suggestion, which is highly beneficial for enhancing the readability of the tables and facilitating a better understanding of the associations between the data for our readers. Following your recommendation, we have added explanations for the naming conventions A, B, C, and D at the bottom of Tables 4 and 5, clearly indicating what each symbol represents. This will allow readers to more intuitively link and comprehend the data presented in the tables. We believe this improvement will make our data presentation clearer and further assist readers in understanding the research findings. Thank you once again for your valuable feedback, which significantly contributes to enhancing the overall quality of our manuscript.

7. In Fig. 6b (1000X), the size of the particles appears to be increasing after reducing the PLGA content from 100 mg to 50 mg. This is contradictive to the DLS measurement and needs to be explained properly.

Response: Thank you very much for your meticulous attention to the selection of electron microscope images, which highlighted an important contradiction we had not noticed. We have re-examined the electron microscope images of the microspheres and carefully reviewed the particle size measurements. Indeed, we found that microspheres prepared with 50mg displayed a slightly larger size under the electron microscope than those prepared with 100mg, contradicting the DSL measurement results. This discrepancy may be due to a reduction in PLGA quality, resulting in uneven, irregular shapes and a porous structure on the microsphere surface, leading to a smaller average size measured by the particle sizer but a larger actual size observed under the microscope. We have added a detailed discussion of this phenomenon to the discussion section of the paper. Thank you once again for your thorough review and valuable comments.

8. The particle size is not demonstrated by Table 6, but mentioned wrongly in the caption and corresponding text in the explanation. Also, the effect of particle size reduction should be correlated with the drug loading rate.

Response: Thank you very much for pointing out the issues with the presentation of the particle size data in Table 6 and for your valuable insights into data interpretation. Following your guidance, we have made the following corrections and additions:

1. We have updated Table 6 to ensure that particle size data is displayed directly within the table, addressing the previous issue where this information was only mentioned in the explanatory text. This change ensures that key data is presented clearly, enhancing the completeness and readability of the table.

2. Regarding the effect of particle size reduction on drug loading efficiency, which you highlighted, your point is indeed correct, and a consistent phenomenon was observed in our experiments as well.

We believe these improvements will help readers better understand the research data and its implications. Thank you again for your meticulous review and constructive suggestions, which have significantly contributed to improving the quality of our research.

9. The drug release data should be explained in more detail. The reason for pH responsive release of BK from PLGA microspheres is not clear.

Response: Thank you for your guidance. We have provided a more detailed explanation of the drug release data in the main text. Additionally, after extensively reviewing the literature and considering various factors such as the physicochemical properties and inflammation levels of the intervertebral disc area, we have discussed the phenomenon of pH-responsive release in the discussion section. This discussion aims to offer readers a more comprehensive understanding and underscores the applicability and scientific significance of our research findings. We believe that these additions and discussions adequately address the reviewer's comments and enhance the quality of our manuscript.

10. From the in vivo data, it appears that the effect of BK/PLGA group is predominantly visible till 1 month and disappears after 2 months. Since the drug release data (Fig. 7e) shows almost 80% release after 1 month, the remaining amount of drug might not be enough to cause significant improvement in the disc health after 2 months. A clear explanation along this aspect may be added in the discussion section.

Response: Thank you very much for your detailed observation and suggestion. Regarding the observation that the effects in the BK/PLGA group are primarily visible within the first month and diminish after two months, as indicated by our in vivo data, we recognize that this is closely related to the drug release data (showing that about 80% of the drug is released after one month, as depicted in Figure 7e). In the discussion section, we have added a clear explanation for this phenomenon, noting that the remaining drug quantity may not be sufficient to induce a significant improvement in the health of the intervertebral disc after two months. Moreover, we discuss potential solutions, such as optimizing the design of the drug carrier to achieve a more prolonged drug release, thereby extending the duration of therapeutic effects. We believe this addition will provide readers with a deeper understanding and highlight the importance of future research directions. Thank you once again for your valuable feedback.

Reviewer #3: In this study, researchers combined clinical data and transcriptome sequencing to identify the involvement of BK in the cellular aging process, and prepared PLGA/BK microspheres through process optimization to save nucleus pulposus cell aging and improve extracellular aging by regulating the B2R and PI3K pathways. The synthesis and metabolism of substrates provide a feasible strategy for delaying IVDD. The author's data supports the proposed viewpoint, but it is recommended to make revisions to the article before accepting the manuscript:

1. There are too many numbers and text in the manuscript images to display clearly. The author should reorganize key data, improve image quality, or display some images that are not the main points of supplementary materials.

Response: Thank you very much for your valuable feedback. We have acknowledged the clarity issues caused by the dense arrangement of numbers and text within the images. Accordingly, we have not only reorganized the key data but also enhanced the image

resolution and optimized the graphic design, ensuring the clarity of numbers and text layout, and preventing overcrowding. We believe these adjustments will significantly improve the clarity of the images and the efficiency of information transmission. Thank you once again for your careful review and constructive suggestions, which are immensely helpful in enhancing the overall quality of our manuscript.

2. In our common understanding, BK is a pro-inflammatory mediator. Will inflammation in the intervertebral disc increase during BK delivery to IVDD? The author should add experimental explanations.

Response: We conducted immunohistochemical staining on paraffin sections from previous experiments to examine changes in inflammatory factors. These latest findings have been added to Section 3.6.5 of the results and are thoroughly discussed in the Discussion section. We are immensely grateful for your suggestion, which not only facilitated this discovery but also encouraged us to conduct a more comprehensive analysis of this phenomenon. This will significantly influence the future research directions of our laboratory.

3. Nanomicrospheres may have a rapid loss probability when injected into intervertebral discs in situ.Suggest the author to add data on the retention time of PLAG/BK nanospheres in vivo.

Response: Thank you very much for your valuable suggestions. Indeed, accurately characterizing the retention levels of nanospheres in vivo using current technologies presents certain challenges. To address this issue, we have conducted detailed in vitro drug release curve tests, hoping that these data can provide useful references for resolving the aforementioned problems. We anticipate that the results of these in vitro experiments will indirectly support our research hypotheses. In the future, we plan to use injectable hydrogels in combination with microspheres to reduce post-injection loss and indirectly demonstrate the retention time of PLGA/BK microspheres in vivo by measuring BK content in the nucleus pulposus. Additionally, we are considering labeling certain elements in PLGA to directly assess the condition of microspheres within the nucleus pulposus, thereby laying a foundation for further research in this field.

4. The discussion section of the manuscript should be revised to enhance the logical coherence of the viewpoints, rather than piling up result data.

Response: Thank you very much for highlighting the area for improvement in the discussion section. We recognize that an effective discussion should not merely compile results data, but more importantly, it should provide a deeper analysis of the significance of these results and a comprehensive answer to the research question. Following your advice, we have revisited and revised the discussion section to enhance the logical coherence of our viewpoints. We have reorganized the content to ensure that each point discussed is closely linked to our findings and that the explanation of the significance of these results in the scientific field and practical applications is more in-depth and clear. We believe these adjustments will significantly improve the quality of the discussion section and the overall value of the manuscript. Thank you once again for your valuable

feedback, which is immensely helpful in enhancing the quality of our paper.

Authors' response to reviewers' comments

Manuscript Number: COMMSBIO-23-4375-T

PLGA/BK Microspheres as a Therapeutic Strategy for Delaying Intervertebral Disc Degeneration

We are grateful to the editor and reviewers for their helpful comments. The manuscript has been revised according to the reviewers' comments and point-by-point response has been made as follows,

Reviewers' comments:

Reviewer #1:

1. The reviewer appreciates the thorough revisions from the authors. Only one issue was discovered: the scale bar for the second row of Fig 5A is missing.

Response: Thank you very much for your positive feedback on our revised manuscript. We greatly appreciate your careful review and constructive comments, which have significantly improved the quality of our work.We have carefully addressed the issue you mentioned regarding the missing scale bar in the second row of Figure 5A. The scale bar has now been added to the figure, and the updated version is included in the revised manuscript.Thank you again for your time and effort in reviewing our work.

Reviewer #2: I appreciate the authors for their sincere efforts in improving the quality of the manuscript. The current version of the manuscript may be accepted for publication.

Response: Thank you for your kind acknowledgment of our efforts to improve the manuscript. We are pleased to hear that the current version is acceptable for publication. Your constructive feedback has been invaluable, and we appreciate your time and consideration throughout the review process.

Reviewer #3: Thank you to the author for their positive response to my review comments. But before the manuscript is accepted, the author still needs to make modifications to the following issues. Otherwise, I do not recommend accepting the manuscript.

We would like to express our deepest gratitude for your thorough and insightful review of our manuscript. Your constructive feedback and thoughtful suggestions have been invaluable in guiding us through the revision process. We have carefully considered each of your recommendations and made the necessary revisions with the utmost attention to detail. Your expertise and commitment to improving the quality of our work have not only enhanced the clarity and precision of our manuscript but also significantly strengthened its overall impact. We truly appreciate the time and effort you dedicated to providing such comprehensive feedback, which challenged us to refine our ideas and presentation, ultimately leading to a more robust and compelling manuscript. It is reviewers like you who elevate the standard of scientific research, and we are profoundly grateful for your contribution. Thank you once again for your unwavering support and valuable input; we hold your feedback in the highest regard and are honored to have had the opportunity to

benefit from your expertise.

1. There is a lot of debate about BK in the study of intervertebral disc apoptosis, and the author has achieved different results through previous data research. Discussions can be appropriately expanded.

Response: Thank you for your insightful comment regarding the debate about BK in the study of intervertebral disc apoptosis. We recognize the ongoing discussion in this area and have expanded the relevant section of our manuscript to include a more detailed discussion of the differing perspectives and our findings in the context of previous research. We believe this addition provides a more comprehensive understanding of the topic.

2. The author verified this method through human body data, and it is very effective. However, the positive rate of BK in fig1A does not seem to be significant.

Response: Thank you for your valuable feedback regarding the presentation of the positive rate of BK in Figure 1A. We understand the importance of clearly conveying this aspect of our data, and we have taken your suggestion to heart. The figure has been revised to ensure that the positive areas are more prominently displayed, making the data easier to interpret. We sincerely appreciate your careful attention to detail, which has helped us improve the clarity of our manuscript.

3. The author can refer to other articles in the journal to improve the presentation of the entire article, and the methods and conclusions should be clearly distinguished.

Response: Thank you for your suggestion to refer to other articles in the journal to enhance the presentation of our manuscript. We have carefully reviewed relevant articles and made adjustments to both the methods and conclusions sections. These revisions have been made to ensure that each section is clearly distinguished and that the overall expression is concise and coherent. We believe these changes have improved the clarity and readability of our manuscript.

4. The author should enhance the discussion mode rather than repeating the results.

Response: Thank you for your insightful suggestion to enhance the discussion section rather than repeating the results. We have thoroughly revised the Discussion section to eliminate any redundancy with the Results. Instead, we focused on a more in-depth discussion of the key findings, particularly those that are contentious or innovative. As a result, while the text in the Discussion is now more concise, it offers a richer and more nuanced analysis. I appreciate your feedback, which has deepened my understanding of these scientific issues during the revision process.

5. Simplify the conclusion.

Response: Thank you for your suggestion to simplify the conclusion. We have streamlined the Conclusion section, focusing on the key findings and their implications while removing any redundant or unnecessary information. The revised conclusion is now more concise and directly highlights the main contributions of our study.

6. I did not see the expression of the core data BK in degenerative mice. Can I supplement this key data to make the entire article more convincing.

Response: Thank you for your valuable suggestion to include core data on BK expression in degenerative mice, which we agree would enhance the overall strength of the manuscript. In response to your comment, we promptly attempted to address this by procuring antibodies from three different suppliers, including Abcam. However, after rigorous testing, we found that due to species-specific limitations, none of these antibodies were suitable for immunohistochemical staining or western blotting in rat tissues. We sincerely apologize for this limitation and regret that we are unable to include the requested data. We appreciate your understanding and your constructive feedback, which has been instrumental in our efforts to improve the quality of our work.

7. The full text images should be significantly modified to better showcase the core content of the manuscript.

Response: Thank you for your suggestion to modify the images in the manuscript to better highlight the core content. In response to your valuable feedback, we have implemented several significant improvements in the latest version of the manuscript. We have increased the resolution of all images to ensure greater clarity and detail. Additionally, we have added appropriate scale bars to all microscopy images, making it easier for readers to interpret the data accurately. Furthermore, we have highlighted the key positive areas in the images, as you recommended, to emphasize the most important findings. To further enhance the manuscript, we have also included a workflow diagram for the Bulk RNAseq and its related analyses in the supplementary material. We believe these enhancements will significantly improve the visual presentation and overall impact of our work.

Reviewer #4: The study by Qiu et al. examines the effects and underlying mechanisms of poly(lactic-co-glycolic acid)/Bradykinin (PLGA/BK) microspheres on intervertebral disc degeneration (IVDD). The manuscript is well-written but lacks important details necessary to understand the rigor of the work.

Thank you for your thoughtful review and for recognizing the merits of our manuscript. We appreciate your comments and have carefully revised the manuscript to address the concerns you raised. Specifically, we have added additional details to clarify the experimental design, methodology, and the underlying mechanisms of the effects observed with PLGA/BK microspheres on intervertebral disc degeneration (IVDD). These revisions aim to enhance the rigor and transparency of our study. We believe that these improvements will help provide a clearer understanding of our research and its contributions to the field.Thank you again for your valuable feedback.

1. The term "whole-gene transcriptome" is confusing. Are the authors referring to targeted transcriptome sequencing or bulk RNAseq sequencing/whole transcriptome sequencing? Please clarify.

Response: Thank you for your valuable feedback. We apologize for the confusion caused by the term "whole-gene transcriptome." This was an oversight in our wording. The correct term should be "bulk RNAseq sequencing," and we have revised the text accordingly to ensure clarity.

2. The methods section lacks a lot of details on the RNA extraction kit, library QC, sequencing technology, and bioinformatics analysis. Specifically:

a) Specify the RNA extraction kit used.
b) Detail any library QC undertaken.
c) Identify the high-throughput sequencing technology and the specific sequencer used.
d) Provide comprehensive information on the bioinformatics steps taken, including tools and their versions, the reference genome used, and any specific QC applied.
e) Explain how the chosen pipeline is suitable for studying cell aging and extracellular matrix metabolism.
f) Are raw RNAseq data being deposited in repositories like GEO?
g) Is the code/pipeline used going to be available?
h) A supplementary figure detailing the bioinformatics steps use might be helpful

Response:

Thank you for your detailed and constructive feedback on the methods section. We have carefully revised the manuscript to address your concerns and have included the following clarifications and additional details:

a) We have specified the RNA extraction kit used in our study in the methods section.
b) Detailed information regarding the library quality control (QC) process has been added to the methods section.
c) The high-throughput sequencing technology and the specific sequencer used have been identified in the revised methods section.
d) Comprehensive information on the bioinformatics analysis has been provided, including the tools and their versions, the reference genome used, and any specific QC measures applied.
e) I In the KEGG pathway analysis, we have highlighted content highly relevant extracellular matrix metabolism with red borders to emphasize their importance.
f) The sequencing data is still under further analysis by our research team, and for data security reasons, it has not yet been uploaded to the GEO database. However, we are prepared to upload it immediately if required; please let us know if this is necessary.
g) We will make the code/pipeline used in our analysis available, and this has been indicated in the manuscript.
h) A supplementary figure detailing the bioinformatics steps has been added to further clarify the process.

We hope these revisions address your concerns and enhance the transparency and rigor of our methods section. Thank you again for your valuable suggestions.

3. In the results section, they state "analysis identified 22 genes with marked differences (P < 0.05)" but do not specify if this is an unadjusted or adjusted p-value. Clarify whether the reported P-value (P < 0.05) is adjusted or unadjusted. This distinction is crucial for interpreting the statistical significance of your findings.

Response: Thank you for your important observation regarding the P-value reported in our results section. The P-value of "P < 0.05" mentioned in our manuscript refers to an adjusted P-value, specifically adjusted using the False Discovery Rate (FDR) method. We understand that this distinction is crucial for interpreting the statistical significance of our findings, and we have clarified this in the revised manuscript.We appreciate your attention to this detail, which has helped us improve the clarity and rigor of our results presentation.

4. The authors mentioned "Among these genes, 12 were notably upregulated (Fig. 3D), while 10 exhibited reduced expression (Fig 3E)" but do not specify in which group. Specify in which group the 12 upregulated and 10 downregulated genes were identified.

Response: Thank you for pointing out the need for clarification regarding the gene expression changes mentioned in our results. The 12 upregulated genes and 10 downregulated genes were identified in the BK intervention group compared to the control group (Group C). We have revised the manuscript to specify this comparison clearly, ensuring that the context of these gene expression changes is well understood.We appreciate your careful review and the opportunity to improve the clarity of our manuscript.

5. In the heatmap (Fig 3C), how do the authors justify that the B2R samples do not show the same expression for genes they claim to be upregulated/downregulated? How have they accounted for sample variability and replicates in their analyses?

Response: Thank you for your insightful question regarding the variability observed in the heatmap (Fig 3C), particularly in the B2R samples. We acknowledge that not all samples may exhibit identical expression levels for genes identified as upregulated or downregulated, which can be due to biological and technical variability.

To address technical variability, we conducted technical replicates, specifically through library preparation replicates and sequencing replicates. By independently preparing multiple libraries from the same RNA samples and performing multiple sequencing runs, we aimed to minimize the impact of technical noise on our results. These replicates were analyzed to ensure the robustness and reliability of our findings. The heatmap represents the average expression patterns observed across these replicates, and while some variability is expected, the overall trends support the upregulation or downregulation of the identified genes. We have added an explanation in the manuscript to clarify the steps taken to account for sample variability and the use of technical replicates in our analysis. We appreciate your comment, which has helped us improve the clarity and rigor of our data presentation.

6. Ensure the legends in Figure 3 are not hidden. The font also seems small making it

difficult to read.

Response: Thank you for your feedback regarding the visibility of the legends and font size in Figure 3. We have taken your comments into account and have made the necessary revisions. The legends in Figure 3 have been clearly detailed, and the font size has been increased to enhance readability. We believe these changes will make the figure more accessible and easier to interpret.We appreciate your attention to this detail, which has helped us improve the overall presentation of our manuscript.

Authors' response to reviewers' comments

Manuscript Number: COMMSBIO-23-4375-C

PLGA/BK Microspheres as a Therapeutic Strategy for Delaying Intervertebral Disc Degeneration

We are grateful to the editor and reviewers for their helpful comments. The manuscript has been revised according to the reviewers' comments and point-by-point response has been made as follows,

Reviewers' comments:

Reviewer #3:

The author has successfully completed the revision of the manuscript. I have no further comments.

Response: Thank you very much for your positive feedback on our revised manuscript. We greatly appreciate your careful review and constructive comments, which have significantly improved the quality of our work. We are pleased to hear that the current version is acceptable for publication. Thank you again for your efforts.

Reviewer #4:

I was pleased to see that the authors were able to address the clarity and description of the methodology in the paper. For the integrity of sharing and reproducible science, authors should deposit RNAseq data in repositories.

Response: Thank you for your valuable feedback. We appreciate your suggestion regarding the deposition of RNA-seq data in repositories for the integrity and reproducibility of the research. We are pleased to inform you that we have uploaded the raw sequencing data to the GEO database, and the accession number is "Series GSE277600". This information has also been included in the revised manuscript. Thank you once again for your unwavering support and valuable input; we hold your feedback in the highest regard and are honored to have had the opportunity to benefit from your expertise.