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ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 10774

Title: Motility patterns of ex vivo intestine segments depend on perfusion mode

Reviewer code: 00227582

Science editor: Ya-Juan Ma

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Motility patterns of ex vivo intestine segments depend on perfusion mode General Comments In this investigation the authors study gastrointestinal motility in a rat model. They determine the effects of different perfusion pressures and perfusion substances on gastrointestinal motility and patterns of motility. They used videos of gastrointestinal motility and fiduciary points to generate colorized maps. They also determined gastrointestinal tissue quality. Motility patterns were distinguished in the colorized maps based on perfusion level and type. The authors conclude that motility experiments should be performed under standardized conditions, both for reproducibility and so that valid comparisons can be made between experiments. This study is of interest since motility is an important indicator of gastrointestinal function. Furthermore, it is of potential importance since there is currently no standard method for characterization of motility. The authors provide colorized maps as a way to standardize motility characterization. This is primarily a qualitative not a quantitative study. There is no quantification of the colorized maps and no statistical comparisons provided. Therefore the conclusions that are made, i.e., that perfusion pressure and characteristics affect gastrointestinal motility and its pattern, are from a qualitative perspective. (This is correct, statistical data were added where applicable. We compared the amount of the different motility pattern for the different media used. Furthermore we compared the total amount of motility pattern. Please see the chapter "Motility patterns dependent on perfusion pressure, storage and perfusion medium" for details.-) I have a few queries. There is little given in the way of description of the colorized mapping process. I would like to see an improved description. There is little description of the software used to measure motility, which is then converted to a color for the map. I would like

to know in detail how this is done, perhaps using a diagram. Are fiducial points on the gastrointestinal surface and the motion of these points analyzed? If so, how are the fiducials selected? Are these some sort of landmarks? Are they virtual? Or are actual markers affixed to the intestine? (An improved description of the mapping process and the software used was added using a diagram (see materials and methods section, figure 4). An improved description of the border tracking was added in the materials and methods section.) There are no tables of quantitative results nor statistical comparisons provided. Is this why the conclusions are somewhat limited? (Statistical data was added where applicable.) I was expecting something might be said about the use of differing perfusion substances and pressures. (Please see <http://www.ncbi.nlm.nih.gov/pubmed/24690290>)– Specific Comments P 4-27 ‘Due to the fact that motility measurement and characterization of motility pattern in the ex vivo system offers a much better feasibility compared to in vivo measurements longer ex vivo times are desirable.’ Please rephrase, don’t really understand this. (We rephrased the sentence.) P 12-27 I don’t really understand MP3. Need to show a figure or characterize better the oral-anal and anal-oral waves. –When are such waves oral-anal and when are they anal-oral? When do other waves get interspersed? How is it determined when the waves are oral-anal and when they are anal-oral? Can we see example of each? How do these relate to the colored maps? (Arrows were inserted into figure 4 (now figure 5) to illustrate the direction of motility waves. Descriptions in the text were improved. We added videos of the different motility pattern to the email.) ‘The stronger the luminal diameter of the intestine decreases at a certain ...’ Not sure if stronger is correct, maybe greater or sharper. P 13-27 I don’t really understand Figure 4. Need a better description of all the details. What are the units of scale? How are these values computed? Why is nothing below a level of about 60 units observable in the colored maps? (An improved description was added. The units of scale are % of the average intestinal diameter. The diameter did not contract below 60 % of its average diameter.) P 15-27 Figure 5 – Are these pie charts the averages for many rats? How many rats? What was the standard deviation? Is there statistical significance between the means for the various solution contents shown? (Statistical data was added to the text. Every pie chart contains data from nine intestinal which were resected from three rats. SEM (of different pressure settings 4-9 cm) was added to former figure 5, now figure 6.) P 17-27 Figure 6 – Are there statistically significant differences?– P 18 - 27 What is ‘superior tissue quality’? ‘Mucosa damage was strongly decreased ...’ How was this measured quantitatively? P 22 - 27 ‘Long-term perfusion with Tyrode usually causes tissue damage.’ What damage? How? (We added an improved description to the text as an indicator of tissue quality we used a decrease in mucosal thickness and structure.) P 23 - 27 ‘Conditions that influence motility can be identified.’ –But statistics are not used to show this, it is qualitative. Quantitative as well as qualitative changes in motility ‘patterns can be evaluated



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ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

This paper describes a new method to visualize the different types of intestinal contractions and compares the effect of different media to perfuse and store the intestinal segments. This is a valuable work to improve the study of intestinal motility ex vivo; however, I consider that this paper should be improved at several points shown below. **ABSTRACT:** The results section does not show the effect of the different media in the rate of motility patterns. A brief description should be given. The final conclusion of the abstract is too general and obvious. A more specific statement according to the results obtained should be more appropriate. (We tried to improve that section.) **MATERIALS AND METHODS:** At the end of the "Motility data interpretation" section, authors stated "For every given dot pair (x-axis) at every given time (y-axis)..." However, in figure 4 it is the opposite: the x-axis is the "time (min)" and the y-axis is "measuring points". (This mistake was corrected.) **RESULTS:** The present paper describes a non-conventional technique to record and analyse the intestinal motility. A more extensive description of the interpretation of the heatmaps showed in Figure 4 would makes easier to the reader to identify the motility patterns that can be observed. (Arrows were plotted into the heatmaps, indicating the direction of motility. A more extensive description was added.) In the legend of this figure 4 it is necessary to state which "measuring point" (y-axis) is in the more oral position: "0" or "32" (The description was added to the legend as well as the text. An additional image was added. It describes the generation of the colorized heatmaps.) Authors present this technique as a pharmacological model to test drugs acting on gastrointestinal motility. I was surprised that authors have not tested any commonly used contractile agent such as acetylcholine or KCl in order to show its response in this model to be compared with other methods from other



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papers. (Please see <http://www.ncbi.nlm.nih.gov/pubmed/24690290>) Authors conclude that “Mucosa damage was strongly decreased using AQIX as storage and perfusion medium”, but they do not describe the morphological changes observed in the histological study after storage and perfusion with the different media. (A more detailed description of the histological changes was added to the results section. The histological examples depicted in the manuscript are supposed to be representative for the media. Other combinations were histologically evaluated too, showing the tendency that combinations with aqix and/or mem lead to a decrease in mucosal erosion.) FIGURE 5: This figure only shows trends to increase or decrease each type of motility pattern depending on the different media used in this study. Why authors have not carried out a statistical analysis to show whether these differences among media could be considered statistically significant? The same can be applied to Figure 6 with the different pressures. How many data (n) are plotted in each histogram? Standard errors of the means (SEM) are absent, so it is difficult to determine the relevance of the results in absence of statistical significance. (Statistical data was added where applicable. Due to the fact that the data was generated by observation of intestinal movements (9 segments for every medium combination, 1' for every pressure setting) we would suggest pooling the amount of contractions observed for one pressure level. If we group the data of one pressure setting as one n and compare the values for medium pressure levels between 4 and 9 cm of water columns as depicted in the pie diagrams, statistical analysis could be performed. The results were added to the paragraph "Motility patterns dependent on perfusion pressure, storage and perfusion medium". SEM (of different pressure settings 4-9 cm) was added to former figure 5, now figure 6) DISCUSSION: If the method described in this paper is compared to that published by Bernard, a brief explanation of the Bernard's method would help the reader to follow the discussion. (An improved description of Benard's method was added to the discussion.) Authors state: “We found that different media compositions similar to food composition in vitro affected the quantity of the motility patterns observed”. However the different nutritional compositions of the three media used are not given in the Materials and Methods section. Furthermore, the differences in the motility patterns obtained with the three media are not discussed according to these different nutritional compositions. (For Tyrode solution nutritional contents were added. The major differences between AQIX solution and MEM medium were listed in the materials and methods section.)



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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Title: Motility patterns of ex vivo intestine segments depend on perfusion mode The paper by Schreiber is interesting and is well written.