BIOEN 326 2014 LECTURE 20: LITERATURE ANALYSIS AND CITATIONS

Overview

In the latter part of Bioen 326, learn knowlege that has only recently been discovered. Because of this, some of the observations have not made it into text books and we need to read original journal articles. Moreover, many of these will appear to contradict each other. For this reason, this is a good time to advance our skills in reading and analyzing literature.

Critical Analysis. This week, we learn the art of **critical analysis** of a journal article, which is the art of determining how certain we are about in the conclusions of the article. This is an important skill because, quite simply, you can't believe everything you read even in scholarly papers. Authors will often point out interpretations of their data that may or may not be true, because these interpretations can help the reader understand the work and can inspire future studies. ("Hey, if that's really true, then in my studies, we would see such-and-such. Wow! I could actually test that!) We refer to the semi-quantitative probability that an interpretation is true as the '**level of certainty**', which I will sometimes refer to as "LOC" for short. Authors will analyze their data and arguments to assign the appropriate level of certainty to their interpretations, and will communicate this to the reader with qualifying words like 'is consistent with', 'suggests', or 'shows'. If you are not trained to interpret these qualifying words, you may believe a conclusion that the authors only wanted you to consider.

The level of certainty is somewhat subjective. Moreover, in their enthusiasm, authors tend to use the highest level of certainty that could be appropriate, and this is not always corrected by the expert reviewers. Should you believe their qualifying words? If you are using the information in a way that effectively bets someone's life, a lot of money, or a lot of someone's time on the correctness of the statement, then you require a high to moderate level of certainty. In these cases, you should analyze the level of certainty for yourself. In this lecture, you will learn to evaluate the methods, results, and both quantitative and logical analysis that led to a conclusion of interest to you. In other cases, you don't need a high level of certainty. For example, if you are just using an article to justify the significance of your work, you really only need to know that the scientific community believes the work, and you can use the authors' words to determine the reliability of their statement, and the words you should use to quality when you paraphrase it. Don't waste your precious time evaluating LOC for this article.

Exercise 1: In an article, the authors propose the interpretation that a new diagnostic test would be more effective than an established test. (For now we will ignore whether this means more accurate, more sensitive, more cost-effective, or all of these.) How certain should you be of this interpretation, for the following situations.

1. You are a doctor wondering if you should use the new diagnostic test instead of the established test for your patient.
2. You are a clinical researcher wondering if you should test the new diagnostic test in clinical trials.
3. You are an engineer deciding whether to design a device to perform this diagnostic test.
4. You are a researcher deciding whether to perform studies to test a hypothesis that would form the basis for this novel diagnostic test.

Level of Certainty Qualifiers

First we will consider the semi-quantitative definitions of the words used to describe the level of certainty of an interpretation.

- ***Highest possible*: Prove** means there are absolutely no alternative possibilities and is only used for a mathematical proof. You should probably never use this in your bioengineering career.
- ***High*: Demonstrates, Indicates, Shows, or no qualifying words (DIS)** means that there are no reasonable alternatives.
- ***Moderate:* May DIS, Strongly Suggests, Seems, Appears** means that there are no likely alternatives. (moderate level of certainty)
- ***Low:* Suggests or may suggest** means that the interpretation given is the most likely; that is, other explanations are likely, but not as likely. **Is consistent with** means no alternatives were eliminated, without evaluation of the likelihood of the one given. This is used for low certainty or to qualify an interpretation before you evaluate its level of certainty.

How do we identify these alternative explanations, and determine their likelihood? The process is very different for an interpretation that arises directly from the data itself, versus interpretations that describe how this direct result may be used to interpret other situations, so we consider these separately.

<u>Exercise 2</u>: Return to exercise 1 and assign LOC words you would need for each case.

Direct vs Indirect Conclusions

A **direct conclusion** is a direct description of an experiment with little or no interpretation. An **indirect conclusion** is an interpretation of one or more of the experiments, analyses, or figures in the article. Consider the following distinctions:

Direct Conclusion	Indirect Conclusion if any of the following:
Refers to a single figure or even one panel of a figure	Is supported by more than one figure panel or figure, so more than one type of experiment.
Describes the same thing that was measured in the figure, or a simple variation (eg force and stress are same if area is known).	Describes an interpretation of a measurement. Describes something that cannot be, or was not, actually measured.
No additional knowledge is needed to draw the conclusion from a single figure.	Additional citations or data are needed to draw the interpretation from the measurement
Statement is obvious from the figure	A logical analysis is necessary to understand how the figure(s) support the statement.
Statement is specific to the experiment described.	Statement generalizes to a broader context.
Often described in past tense	Stated in present or future tense, because

Because of the need for citations and logical analysis to support indirect conclusions, they are often left for the discussion, while direct conclusions form the bulk of the results section. However, the indirect conclusions are what gives meaning to the data, so a paper is much more interesting and easier to understand (since interest leads to understanding) if the results section also includes some indirect statements. In some cases, the results section will provide the indirect statements with a low level of certainty (this data suggests that…), to motivate the next section. They don't need a high level to justify another experiment, so this lets them tell a story without including citations and arguments, which should wait until the discussion. Then the discussion can finally bring everything together and argue a higher level of certainty. Don't be fooled by the low LOC in the results in such cases.

Examples of direct conclusions:

1. "Increase of radius and decrease of wall thickness were most marked as distending pressures increased from 5 to 80 mm Hg." (in the results of Wolinsky 1964) and "Circumferential waves and folds in elastin lamellae diminish and interlamellar distances decrease uniformly throughout the wall as distending pressures increased from 5 to 80 mm Hg" (in the caption of figure 7 of Wolinsky 1964) (Wolinsky and Glagov 1964)
2. "The UTS values of all of the scaffolds were greater than … or comparable to the UTS of human ACL … (Supplementary Fig. 3)" (Freeman, Woods et al. 2007)

Examples of indirect conclusions:

1. "…at physiological pressures the aortic media functions as a "two-phase" material; circumferentially aligned collagen fibers bear the tangential stressing forces while the elastin net distributes the stressing forces uniformly throughout the wall". (the last sentence of the introduction, where people often summarize what they show, of Wolinsky 1964 (Wolinsky and Glagov 1964))
2. "The braid–twist scaffold studied was found to be a promising construct for tissue engineering of the ACL." (Freeman, Woods et al. 2007)

Assessing Level of Certainty for Direct Conclusions.

For direct conclusions, you only need to consider whether the proper controls were done to draw the conclusion, and whether the differences observed to draw these conclusions are statistically significant.

<u>Statistical significance</u>:

1. Do figures have error bars? What do they mean? Ideally, the error bars should represent the standard deviation between the results for multiple experiments. However, it may involve multiple samples on the same day. If so, were similar results seen on other days? Minimally, make sure they have some measure of the error. Better yet, scan the figure caption, the related text, and the methods to understand the source of error and decide if you agree with it. That is, do the error bars include all likely sources of error in this experiment?

2. If they draw a conclusion from a difference between two values, is the difference more than can be explained by random error? If the answer is obvious, then no statistical test is needed. That is, if the error bars of the two data points are nowhere clear to overlapping, there is no need to do the analysis. However, if the error bars overlap or come close to overlapping, a statistical test is needed. Often, the result is given as a p-value, and $p < 0.05$ is considered high level of statistical certainty that the difference in the two measurements cannot be explained by random error. Minimally, decide if a statistical test is needed and if so, did they do it and what did they learn. Better yet, assess whether the test is appropriate for this case.

<u>Controls</u>:

1. Negative controls. A negative control is designed to show that your measurement is actually due to what you think, rather than some random other thing. This is critical for biological measurements, and indicates that your assay is specific. For example, if you are measuring the presence of a protein or gene with an antibody or hybridization nucleotide, you need to show that you don't get nonspecific binding. However, it is usually unnecessary with a mechanics test of a macroscale object. If you fasten a material into a testing device, and measure stress and strain until the material fails, there is little besides the material than could cause the measurement. A negative control would simply show that there is no stress if there is no material, but this is a given if the device is functioning properly, and would not be shown in a figure.
2. Positive controls. A positive control is designed to demonstrate what response you would expect if the answer is 'yes'. These are critical to trouble shooting. That is, if you get a negative result in your experimental condition, how do you know it gives you a 'no' answer, rather than that the experiment didn't work? However, they are often not included in publications unless you are demonstrating that the answer was no. If instead you get a response with your experimental condition, you may not need a positive control. Again, mechanics experiments may also not have a relevant positive control.
3. To determine if the needed controls are included, ask what else could have caused the result they observed, and if you can think of anything, see if they did any controls to make sure that was not it.
4. Example 1. Consider a hypothetical example in which the authors detected that protein A, but not protein B, in a cell 30 minutes after mechanical stimulation. What controls do they need to conclude that mechanical stimulation induced expression of protein A but of not protein B?
    a. They need to make sure that protein A would not have appeared without the stimulation. So they need a negative control in which the cells were put in a device to be stimulated, with all same the buffers, for the same time, but were not actually subjected to the mechanical stimulation. If the figure shows a range of stimulation values (eg range of force, range of amplitude of cyclical strain, etc), and the response changes from off to on over this range, then an additional negative control is not needed, since the low part of the range serves as a control showing that the response would not occur for stimulations below a threshold

value. In fact, this is the best negative control, since conditions are most identical other than the strength of stimulation. However, if they conclude that the increase is due to some mechanism, other controls would be needed.

b. They need to make sure they would have detected protein B if it had been induced. This ensures that their reagents are working, their assay is sufficiently sensitive, and the time frame for induction is appropriate. The best positive control would be an alternative type of stimulation known to induce protein B.

As long as a figure includes these controls, and the statistical issues are satisfied, the authors can draw the direct conclusion that mechanical stimulation of the type used in this assay induces protein A but not protein B. Without the controls, the statement would need to read something confusing and useless such as "Cells subjected to mechanical stimulation reacted at a measureable level to our probe for protein A, but not to our probe for protein B."

5. Example 2. Figure 1 is a figure from my graduate work. I might describe this figure with words such as "More bacteria bound via FimH to mannose at 0.4 Pa than at 0.01 Pa." Note that I use a high level of certainty, because I used no qualifying words. Because this is a direct conclusion, we only need to address the statistical analysis and the controls. I did not need to include a quantitative statistical analysis because the difference in the values at 0.4 vs 0.01 was so much greater than error. The caption notes that the result was reproducible, but it would have been even better to include averages and standard deviations from experiments on different days. The control with gal-BSA demonstrates that adhesion is specific to mannose. The experiment should also have a negative control with a strain of bacteria that were identical except that they did not express FimH. While we had shown in previous publications that this strain of bacteria only bound to mannose through the adhesin FimH, we should have included a negative control that shows that in these conditions, an isogenic strain that lacks FimH also showed no adhesion. This would avoid the need to include a citation to support a direct statement. In summary, the high level of certainty is warranted, but the figure could have been improved to make it even higher, and/or to make it easier to understand.

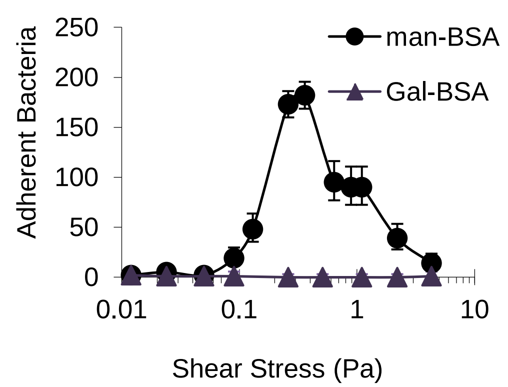

Figure 1. Number of *E. coli* bacteria adhering to surfaces coated with mannosylated bovine serum albumin (man-BSA) or galactosylated BSA (gal-BSA), when bacteria are injected at a flow rate that creates the indicated wall shear stress. Similar results were observed in three independent experiments.

Assessing Level of Certainty for Indirect Conclusions.

For indirect interpretations, you need to consider a wider range of issues. You still need to address the level of certainty of the direct conclusions that support the indirect conclusions. In addition, you need to address what additional information is required to expand the direct conclusion to the more broad and informative indirect conclusion. We usually perform the experiments in order to address the question answered by these indirect conclusions, and we string together experiments that allow us to have a higher level of certainty for this significant indirect conclusion than we could with one experiment alone. We also add information supported by citations, and our own logical analysis to address alternative explanations.

To make your own level-of-certainty analysis, or even to understand the authors' analysis, you will need to ask about what alternative explanations could be consistent with the direct conclusions but not with the indirect ones. For example, in the Freeman paper, the authors test a braided and twisted collagen scaffolds to determine if they have the right mechanical properties for scaffolds for tissue-engineered ACL (a ligament in the knee). They measure the UTS of the scaffolds and ACL, and their direct conclusion is that the two are consistent. To draw the indirect conclusion that "The braid–twist scaffold studied was found to be a promising construct for tissue engineering of the ACL", what alternatives should they consider? They need to address logic and prior literature about what is important for tissue engineering of ACL. Logic and experience suggests the importance of Young's modulus, as well as issues of biological compatibility such as toxicity, allergic responses, degradation in the body. We will scan the paper to see whether and how the authors address such issues, as well as whether they addressed issues we might not have thought about. They may include additional measurements or they may cite prior work. For example, ligaments normally have collagen, so the authors can argue that toxicity is not an issue. However, the authors should probably include measurements of Young's modulus, since it is important to show that the exact same scaffolds that have the consistent UTS also have a consistent Young's modulus. It should be noted that their use of the term "promising" indicates only a moderate level of certainty, so they do not need to address all alternatives.

Note: If you are doing research yourself, you should train yourself to use the correct LOC words when you orally describe your results to your mentors and advisors, as well as in lab notebooks, and in formal write ups. If a junior person accidentally overstates the level of certainty, this is an invitation for senior lab members to challenge the controls, statistical analysis, and logical analysis. The junior person often feels attacked, thinking, 'but I haven't gotten there yet; I was going to raise these issues myself'. However, their words suggested that they had completed the analysis. By choosing your LOC words more carefully, you will feel more supported and gain the respect of your mentors.

Citations, Paraphrasing, and Quotations

When you talk about what was said in a paper, it is necessary to use proper citations or quotations to distinguish your opinions from previously published facts and opinions. Failure to cite correctly often results in plagiarism. This is considered academic misconduct for a class and scientific misconduct for a grant proposal or a published article. If any of this is new to you, you may have turned in work that is plagiarized in the past without realizing it, and gotten away with it because instructors knew that you had not yet learned this skill, or simply did not catch your mistake. This is a learning objective of this lecture, so you will be graded on your ability to apply this correctly, but will not be accused of academic misconduct if you make mistakes. In later courses, however, you may be accused of academic misconduct if an instructor feels you were trying to get credit for ideas that are not your own.

1) **No citations are needed**:
    a. Wendy's rule of textbook: You can paraphrase things found in undergrad textbooks without a citation (This is no rule of Wikipedia!)
    b. If you are expressing ideas that are truly your own or are describing data (experiments or simulations) that you are publishing here for the first time.
2) **Paraphrasing** means you use your own words to restate someone else's ideas or data.
    a. Paraphrasing is almost always better than quoting another article, because paraphrasing allows you to tailor the words to efficiently focus on the ideas you need to get across, using the context you have already set for your reader.
    b. When you paraphrase, you must cite the original article(s) unless it satisfies #1 above (no citations needed).
    c. How do you know which article or articles to cite? You should always cite the article(s) that help your reader learn as quickly as possible how the information is known so he or she can read them and determine the LOC for him or herself. If the statement is the conclusion drawn in one or a few papers, you should cite these original articles. If the statement arises from a large number of articles, then you should cite one or more reviews that summarizes how this conclusion is supported by those many articles. What you do not want to do is to cite a paper that paraphrases someone else in the same manner as you did, because your reader will then have to follow a citation trail.
    d. You shouldn't cite Wikipedia articles or other web sites to substantiate a fact in a scholarly work, because web sites are in flux and usually not peer-reviewed. Wikipedia is a great way to learn things quickly, and there is no need to cite this knowledge at all if it becomes clear it is common knowledge (ugrad text rule-of-thumb). However, you need to follow the Wikipedia citations or search the scholarly literature if you need to use what you learned on Wikipedia in your own scholarly work.
3) **Direct and Indirect quotes** involve using the author's words. A direct quote is an exact quote and must be placed in quotation marks. An indirect quote is the use of very close wording, and must be attributed to the source with phrases such as "the authors argued that…" or "he said that" instead of placing the material in quotes. (Note: direct and

indirect quotes have nothing to do with our discussion of LOC for direct and indirect conclusions).

   a. Quotes of either type are used when you want to emphasize that the statement is the opinion of that author, or when the authors' original wording is important to your point.
   b. You must always include the citation regardless of how well known the idea is. You have used the authors words, so failing to cite is clear and unequivocal plagiarism. There is no point in using quotes if you don't allow the reader to identify the original author, since you are using them to emphasize the authors' wording or opinions.
   c. Examples:
      i. Freeman *et al* noted that they anticipated that PEGDA would not hinder cellular proliferation (Freeman, Woods et al. 2009). (indirect quotes)
      ii. Coste et al stated "Piezos are components of MA cation channels" (Coste, Mathur et al. 2010). The lack of any qualifying words indicates that the authors made this statement with a high level of certainty. (direct)
      iii. Huang et al claimed a high level of certainty when they wrote "this finding indicated that MSCs were viable in CM-, but deposited very little ECM. (Huang, Yeger-McKeever et al. 2008)"
   d. It is rare that the other person's exact words will fit seamlessly into your text. Unless it is necessary to draw attention to the choice of words used by the source, quoting will usually sound awkward and lazy. Most scientific articles rarely use quotes, and you should generally not use them in your scientific scholarly work. ***For this class, you should only use quotes to describe the author's claimed level of certainty***.

Summary

- Qualifying words indicate level of certainty (LOC) as follows: No qualifying words or DIS means high certainty (no reasonable alternatives), may DIS or strongly suggests or seems means moderate certainty (no likely alternatives), and suggests or 'is consistent with' means low certainty (alternatives possible or not considered).
- Direct conclusions are a description of the data and have a high level of certainty if the controls are statistics demonstrate reliability of the experiment.
- Indirect conclusions are an interpretation of the data and depend require additional analysis, citations, and/or experiments for a high level of certainty. Most indirect conclusions build on the direct conclusions of the data, so they also rely on the LOC of the direct conclusions.
- The key to conducting your own LOC analysis is to ask what alternatives could explain the data, and then consider whether controls, statistics, analysis, citations, and other experiments eliminated these.
- Use quotations to describe the author's level of certainty, but use paraphrasing in general for your scholarly work; in both cases, cite the appropriate original work.

References

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