

MSE 471: Tips for experimental design

Step 1. Generate a research question and hypothesis

A good experimental design starts with a thorough understanding of the subject that you are studying. After identifying the independent variables affect the system and how they are related, you can formulate a specific research question and hypothesis. Your research question should be clear, analytical (rather than descriptive), specific and focused. From the research question follows the hypothesis, which needs to be specific and testable.

Examples

Specific research question: How do non-crosslinked alginate polymers affect cell viability?

Hypothesis: I hypothesize that non-crosslinked alginate does not negatively affect the viability of primary human fibroblasts.

Step 2. Design a controlled experiment

The hypothesis that you proposed needs to be tested in a carefully controlled experiment to make sure that you are able to obtain the data needed to answer your research question. This requires a strong understanding of the system you are studying. There are a number of important aspects to consider:

- 1. Keep the experiment as simple as possible**
- 2. Systematically and precisely manipulate the independent variable(s)**
 - Always make sure to vary only one parameter at a time so you know what variable is causing the effects that you measure
 - Identify the (clinically/biologically/practically) relevant range in which you want to vary the parameter of interest
(e.g. the concentration range of alginate that you are going to test)
- 3. Include negative and positive controls**

To confirm the validity of your experimental setup, each experiment needs proper controls. The negative control is a control group that is not exposed to the 'treatment', e.g. cells cultured in medium without alginate.

The positive control is a control group that is exposed to some other treatment that is known to produce the expected effect.
e.g. cells cultured with a cytotoxic agent such as DMSO which will induce cell death
- 4. Include replicates (technical replicates and/or biological replicates)**

To confirm that what you are measuring is valid and reproducible, you need replicates. There is a distinction between technical replicates (e.g. preparing three wells with the exact same cells and alginate solution to measure cell viability three times) and biological replicates (using three batches of cells (e.g. from 3 patients) that are cultured with alginate solution to measure cell viability).
- 5. Precisely measure the dependent variable(s)**

Identify a method to measure your readout parameter with precision
- 6. Control any potential confounding variables**

A confounding variable is a variable that has an impact on your experiment, but you are not interested in. They have to be kept constant, otherwise they may lead to wrong conclusions. For instance, different cell lines have different proliferative capacity. Hence, if you are interested in the effect of the hydrogel properties in cell proliferation, you have to use the same cell line for all the different conditions.