

Serum-Free Alternatives for Culturing Pilot Whale Fibroblasts

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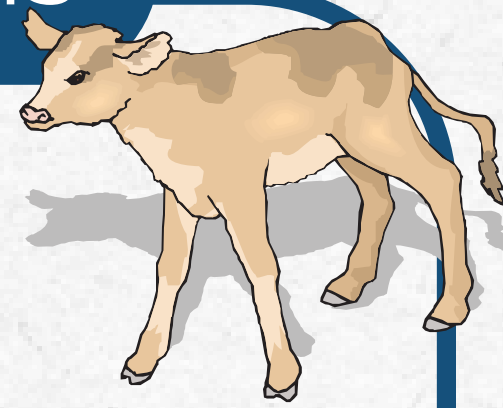
Background and aims

Cell culture traditionally relies on **Fetal Bovine Serum (FBS)**, which is problematic for the following reasons¹:

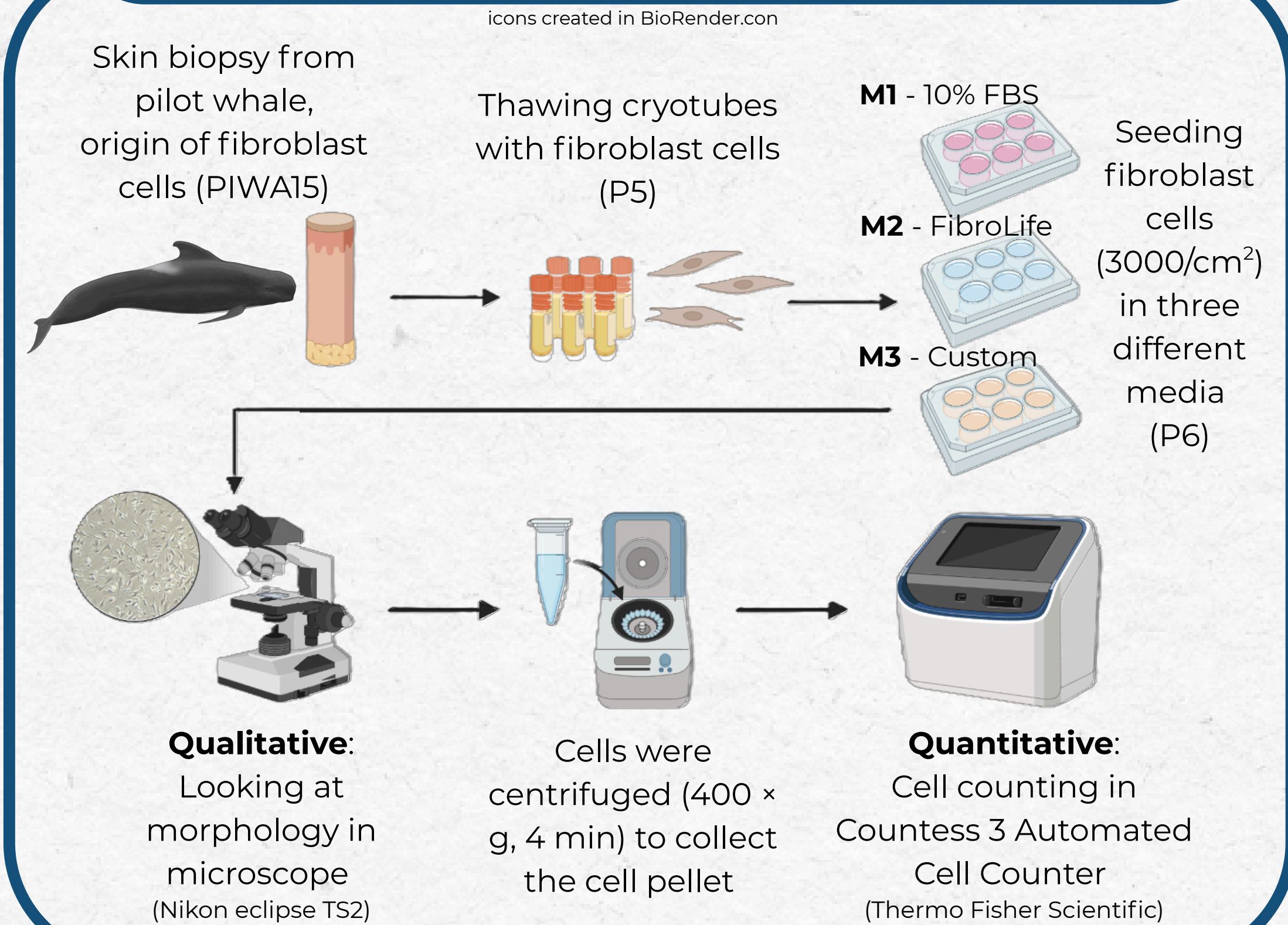
- **Ethical controversy** as it is harvested from bovine fetuses (calves) during the slaughter of pregnant cows.
- **High batch-to-batch variability** that undermines experimental reproducibility.
- **Risk of contamination** from viruses, mycoplasma, endotoxins.

This project aimed to explore **animal-free alternatives**² by:

1. **Developing** a custom chemically defined medium based on existing literature^{3, 4}.
2. **Testing** this custom medium (**M3**) on pilot whale fibroblasts alongside a commercial serum-free medium, FibroLife⁵ (**M2**), and a standard 10% FBS control (**M1**) to compare growth performance and cell morphology.



Workflow and experimental setup



Results

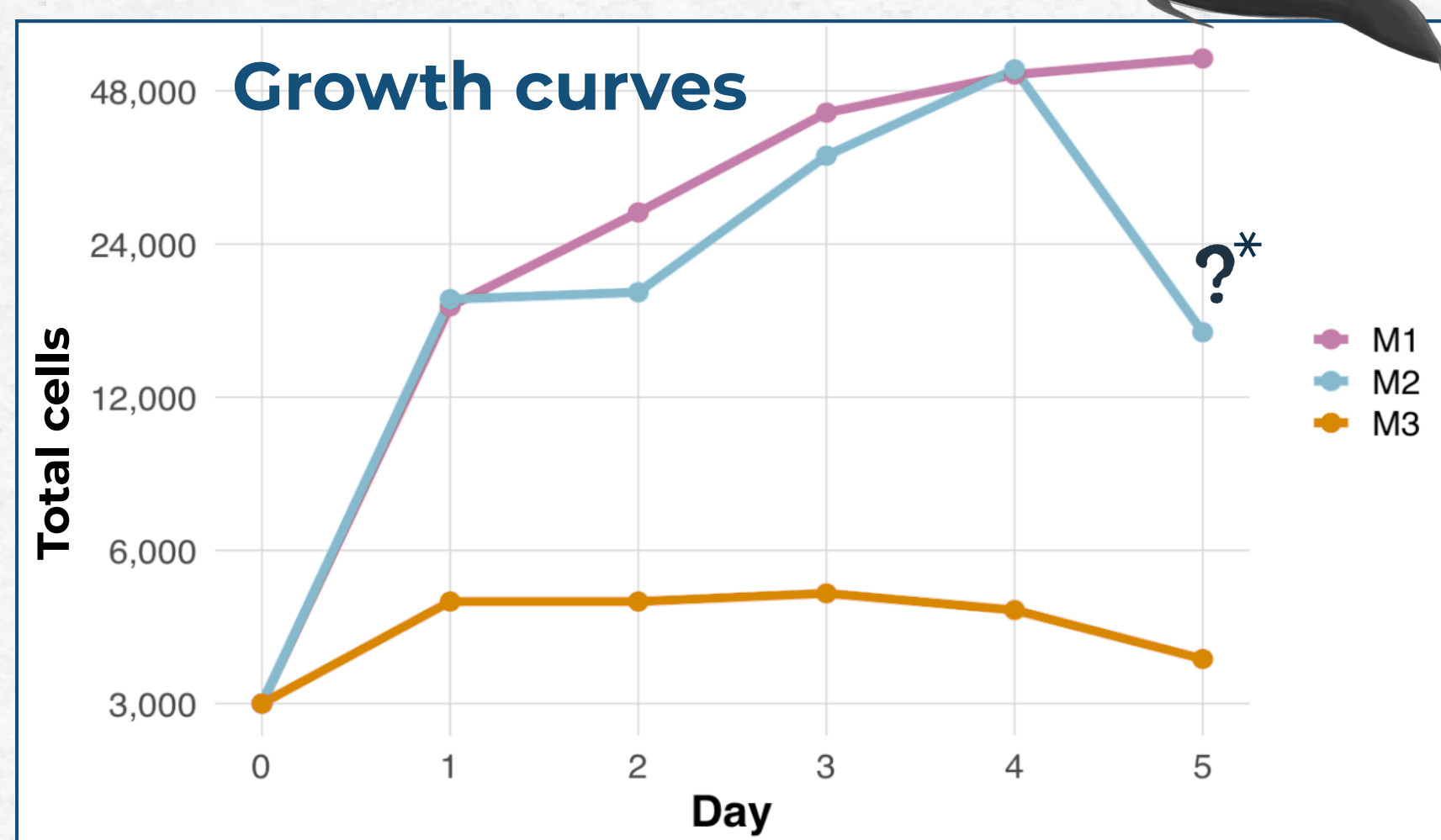


Figure 1: Growth curves for pilot whale fibroblasts cultured in three media (M1: 10% FBS, M2: serum-free FibroLife, M3: custom defined medium). M1 and M2 supported strong early proliferation (*the sudden drop for M2 is a possible outlier, see below), while M3 showed minimal growth.

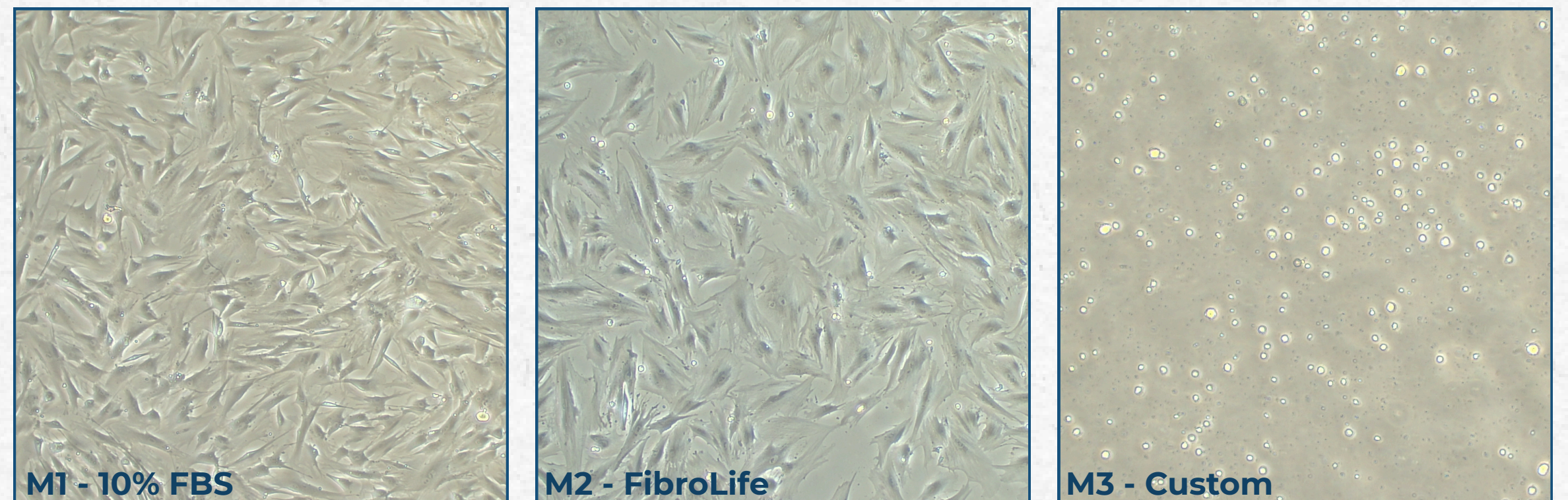


Figure 2: Morphology of pilot whale fibroblasts on day 4, imaged at 4x magnification using a Nikon Eclipse TS2. M1 and M2 show typical elongated, spindle-shaped fibroblasts, while M3 displays rounded, poorly attached cells indicative of limited growth and reduced viability.

Optimal pH for cell culture is 7.2- 7.4⁶.
pH measured in media:

- M1 - 7.35
- M2 - 7.52
- M3 - 6.99

Conclusion

- ✗ M3 did not support fibroblast growth, and the exact cause remains unclear due to its exploratory, first-draft formulation. Possibly influenced by its low pH (6.99).
- ?* The day 5 drop in M2 cannot be interpreted reliably, as the experiment lacked replicates and it may reflect technical variation or handling error.
- \$ FibroLife (M2) matched FBS-based (M1) growth up to day 4, but is ~7x more expensive than FBS, which is an important practical limitation.

Replacing FBS is possible - but not with M3 (yet)

Future work

- **Optimize M3** using modular testing.
- Check **species-specific needs** (FibroLife = human-focused).
- Compare **long-term performance** of FibroLife vs. FBS.
- **Enzyme activity assays** to test whether key enzymes activate in FibroLife as in FBS.

