

A blocked split-strip-plot experiment to detect the influential steps in a cell-based bioassay

Alexandre Bohyn

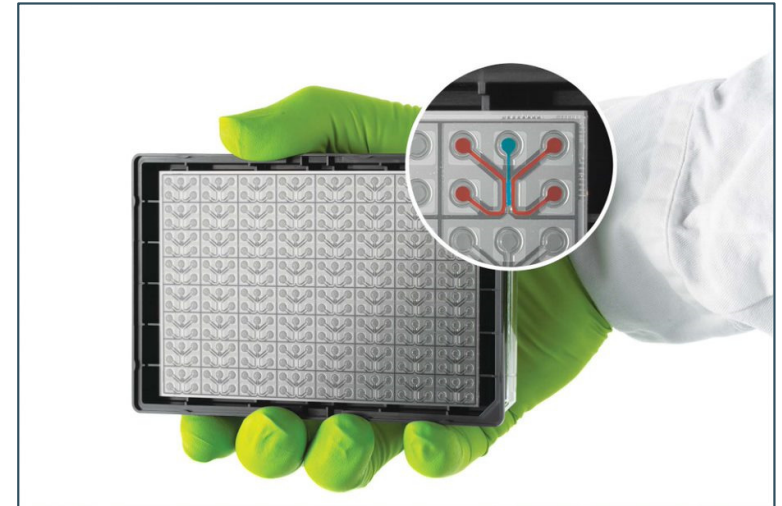
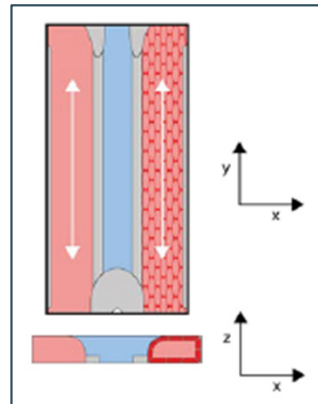
Joint research with: Eric Schoen & Peter Goos (KU Leuven)

Collaboration with: Chee Ng, Kristina Bishard, Manon Haarmons and Sebastian Trietsch (Mimetas BV)

2022 ENBIS conference

Introduction

- Collaboration with Mimetas BV (Leiden, The Netherlands)
- They manufacture the **OrganoPlate®**:
 - 3D culture plate with several chips
 - Epithelial cells are grown on a gel
 - Mimics intestinal cells
 - Used for permeability studies



How is the experiment conducted?

1. Preparing the **gel**
2. Loading the gel inside the **chips** of the plate
3. Once set, seed the **cells**
4. Once grown, the plate is ready
5. Measure fibrosity

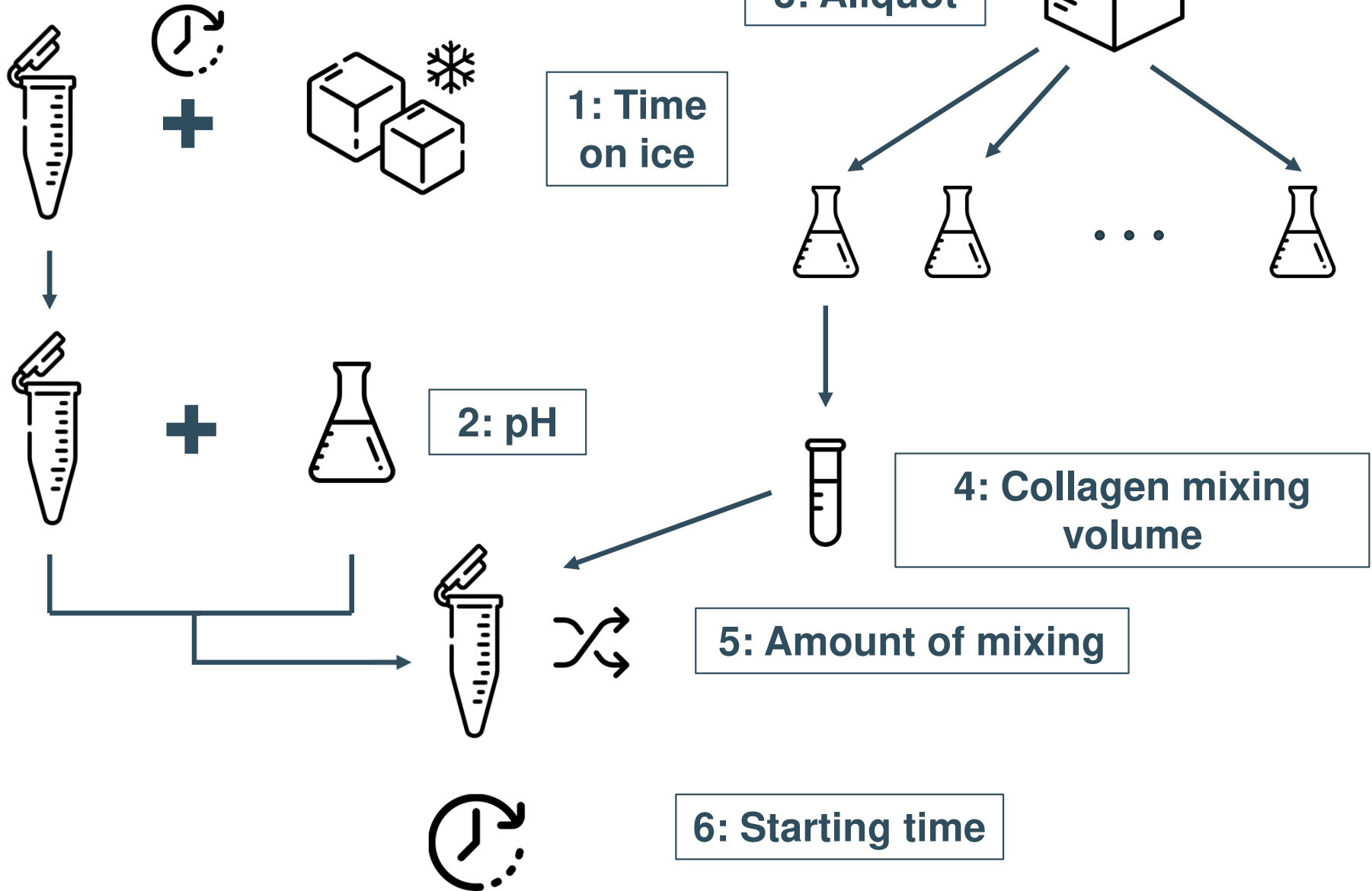


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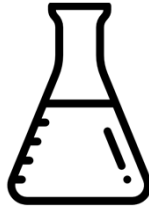
We are only looking into the protocol of **the first two steps**

We want to identify the **potentially influential steps** of the protocol

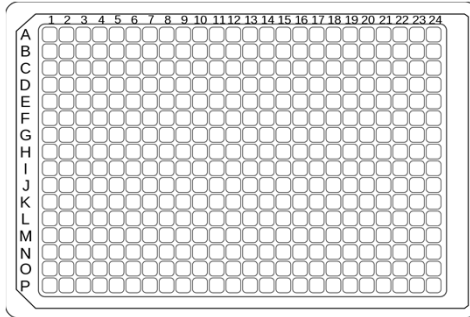
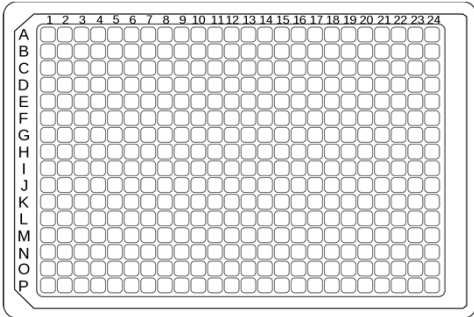
Preparing the gel



Finalizing the plate



7: HBSS Mg/Ca levels



8: HBSS removal

Factors identified

Stage	No.	Factor	Settings	
Gel preparation	1	Time on ice (min)	1	30
	2	pH of solution	7,1	8,3
	3	Aliquot	Late	Early
	4	Collagen mixing volume (μ l)	100	300
	5	Amount of mixing	20	50
	6	Starting time (min)	10	60
Loading	7	HBSS Mg/Ca levels	-	+
	8	HBSS removal	No	Yes

Split-plot structure

- Factors can only be varied by **column**
→ only 8 runs per plate
- 4 plates available: **32 runs**
- Maximum of two plates per week:
split-plot structure

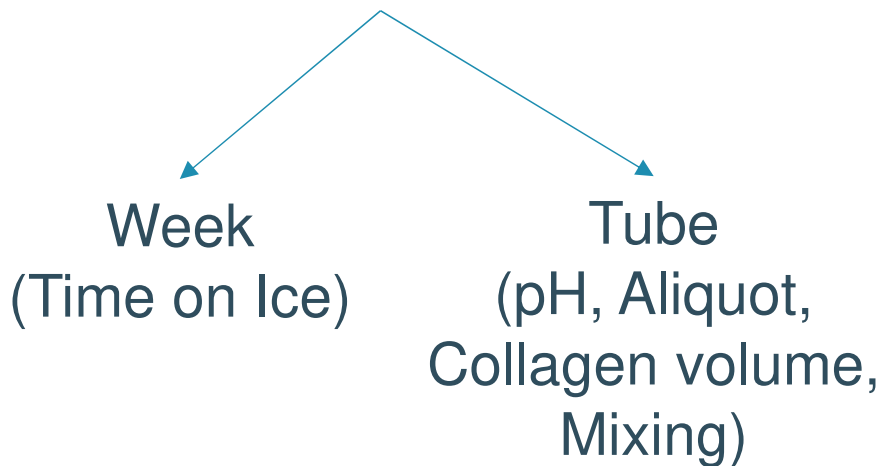


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Week	Plate	Time on ice (min)	Start time (min)
1	1	30	60
1	2	30	10
2	1	1	60
2	2	1	10

Another split-plot structure

- Logistic reasons:
8 tubes for each week
- 4 factors are varied between the tubes :
split-plot structure



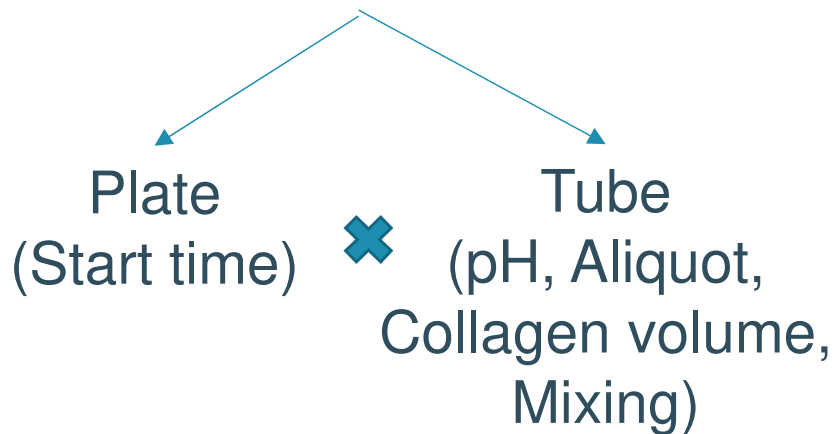
Example for week 1

Tube	pH	Mixing	Aliquot	Col. vol.
1	7.1	50	Early	100
2	7.1	20	Early	300
3	8.3	50	Early	100
4	8.3	20	Early	300
5	7.1	20	Late	100
6	7.1	50	Late	300
7	8.3	20	Late	100
8	8.3	50	Late	300

Tubes to fill the plates



- Gel is prepared inside individual tubes
 - 8 tubes per week = 16 columns to fill with 8 tubes → **one tube is used for two plates**
 - Tubes are **crossed** over the plates
- **Strip-plot** structure



	Tube															
Plate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	X	X	X	X	X	X	X	X								
2	X	X	X	X	X	X	X	X								
3									X	X	X	X	X	X	X	X
4									X	X	X	X	X	X	X	X

Final design

8 factors studied:

- *Time on Ice* varied over the **weeks**
- *Start time* varied over the **plates**
- *pH, Aliquot, Collagen, Mixing* varied over the **tubes**
- *HBSS removal, HBSS Ca/Mg* varied over the **columns**

		Week 1: Time on Ice: 60 min								
		Tube 1	Tube 4	Tube 5	Tube 8	Tube 10	Tube 11	Tube 14	Tube 15	
Factors										
pH		7.1	7.1	8.3	8.3	7.1	7.1	8.3	8.3	
Aliquot		20	50	20	50	50	20	50	20	
Collagen mix. Vol.		Early	Early	Early	Early	Late	Late	Late	Late	
Plate	Start time	Mixing								
			100	300	100	300	100	300	100	300
1	60		Yes, +/+	No, -/-	No, +/+	Yes, -/-	Yes, +/+	No, -/-	No, +/+	Yes, -/-
2	10		No, -/-	Yes, +/+	Yes, -/-	No, +/+	No, -/-	Yes, +/+	Yes, -/-	No, +/+

Final design

Time on Ice: 1 min										
	Factors	Tube 1	Tube 4	Tube 5	Tube 8	Tube 10	Tube 11	Tube 14	Tube 15	
	pH	7.1	7.1	8.3	8.3	7.1	7.1	8.3	8.3	
	Aliquot	20	50	20	50	50	20	50	20	
	Collagen mix. Vol.	Early	Early	Early	Early	Late	Late	Late	Late	
Plate	Start time	Mixing	100	300	100	300	100	300	100	300
1	60		Yes, +/+	No, -/-	No, +/+	Yes, -/-	Yes, +/+	No, -/-	No, +/+	Yes, -/-
2	10		No, -/-	Yes, +/+	Yes, -/-	No, +/+	No, -/-	Yes, +/+	Yes, -/-	No, +/+

Time on Ice: 60 min										
	Factors	Tube 2	Tube 3	Tube 6	Tube 7	Tube 9	Tube 12	Tube 13	Tube 16	
	pH	7.1	7.1	8.3	8.3	7.1	7.1	8.3	8.3	
	Aliquot	50	20	50	20	20	50	20	50	
	Collagen mix. Vol.	Early	Early	Early	Early	Late	Late	Late	Late	
Plate	Start time	Mixing	100	300	100	300	100	300	100	300
3	60		No, +/+	Yes, -/-	Yes, +/+	No, -/-	No, +/+	Yes, -/-	Yes, +/+	No, -/-
4	10		Yes, -/-	No, +/+	No, -/-	Yes, +/+	Yes, -/-	No, +/+	No, -/-	Yes, +/+

Structure summary

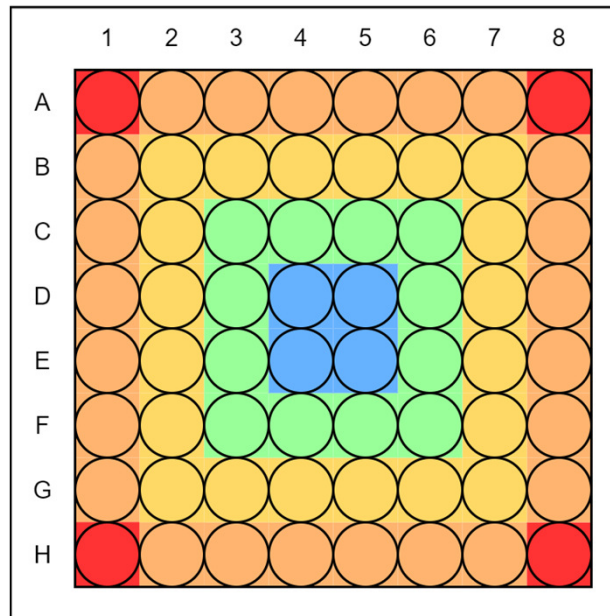
- Split-strip-plot structure
 - 8 factors:
 - 1 randomized between weeks
 - 1 between the plates
 - 4 between the tubes
 - 2 between the columns
 - Each randomization = specific random error term
- 31 factorial effects in 4 strata

Error stratum	Number of effect estimated
week	1
plate	2
tube	14
unit	14

→ not all effects estimated with same precision

What about the blocks?

- There might be edge effects
- To account for that, we balance the factor levels over the columns on the plate
- We create **8 blocks for the 8 columns** of a plate



Aliasing of the terms

- No **main effects** aliased with **two-factor interactions**
- 4 **two-factor interactions** not aliased with other **two-factor interactions**
- 9 pairs of aliased **two-factor interactions**
- 2 triplets of aliased **two-factor interactions**

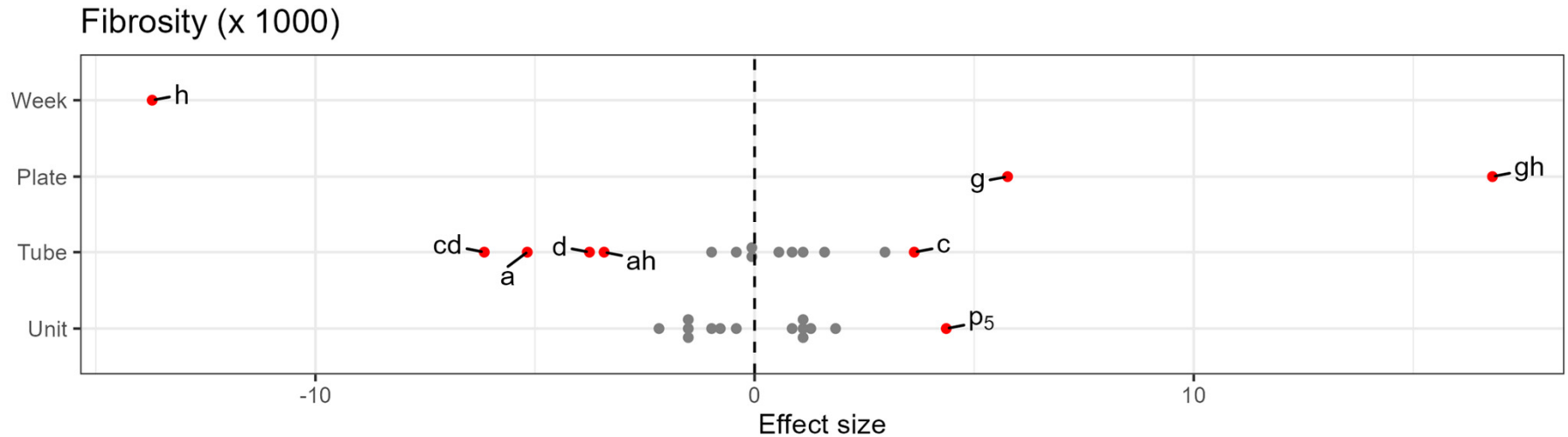
- We can link each alias string to its corresponding error stratum

Building the model

1. Estimate 31 coefficients
2. Separate them by error stratum
3. Compute a robust estimator of the standard error for the two strata with 14 *d.f.*
4. Determine active effects
5. Build a final model using the active effects

Modelling fibrosity

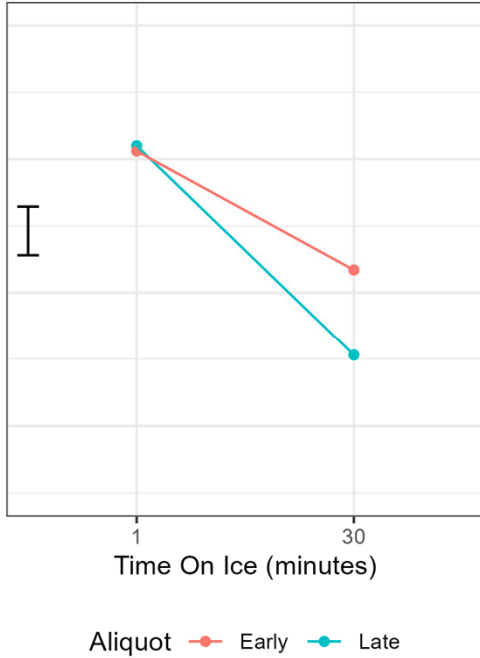
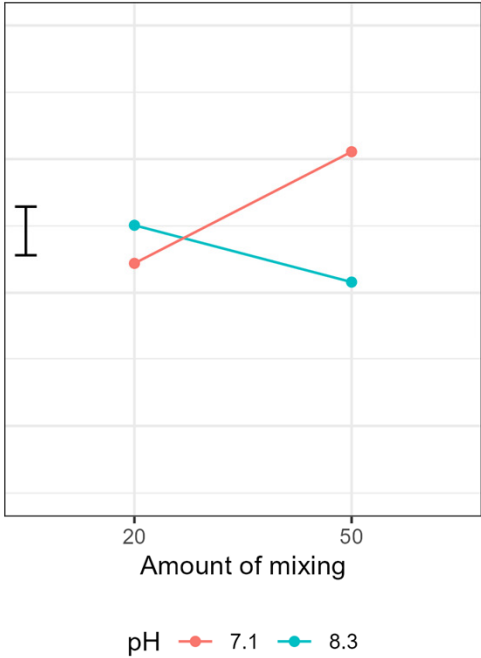
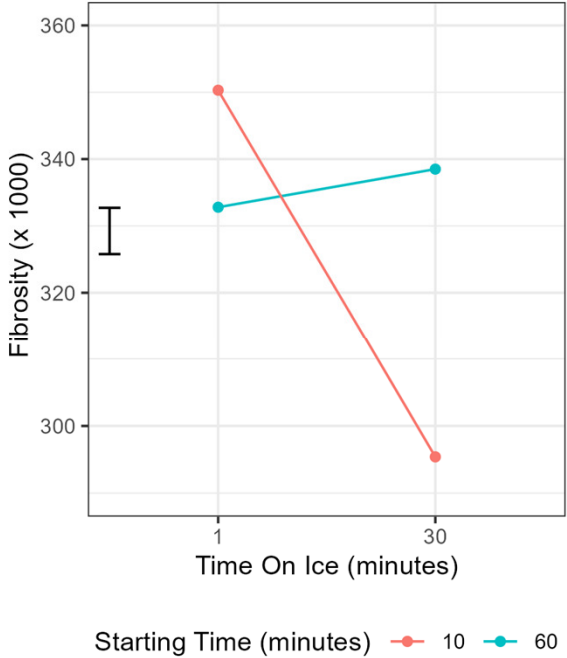
- Identify the factors that have an effect on fibrosity



Letter	<i>a</i>	<i>c</i>	<i>d</i>	<i>g</i>	<i>h</i>	p_x
Factor	Aliquot	Mixing	pH	Start Time	Time on Ice	Column differences

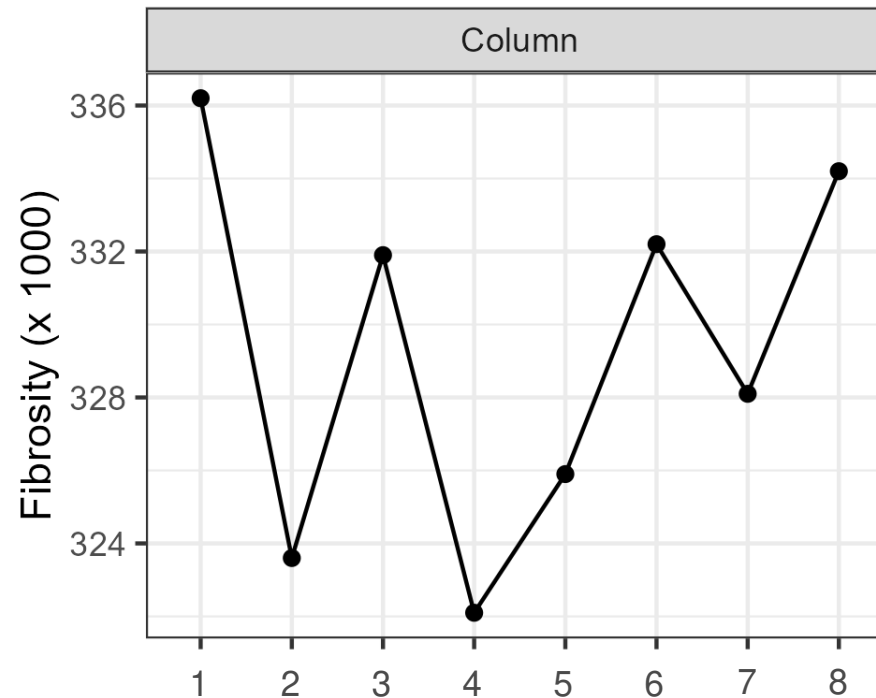
Effect plots

3 active interactions detected



Column effect

High variation between columns and potential edge effects



Conclusions

- Experiment structure was dictated by the experimental conditions
- Even simple experiments can have a complex error structure
- Blocking was essential to detect the column effects
- Multiple iterations needed → initial designs exposed logistic issues
- Mimetas very satisfied with DOE → **better protocol implemented**

Thank you !