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Collagen and PDMS Scaffolds for C2C12 Muscle Tissue Cell Line



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Introduction

Scaffolding, traditionally made of polymeric biomaterial, is used to provide the structural support for cell attachment and eventually tissue development. Depending on the nature and properties of the cells, the scaffold properties need to change.

Collagen Scaffolds:

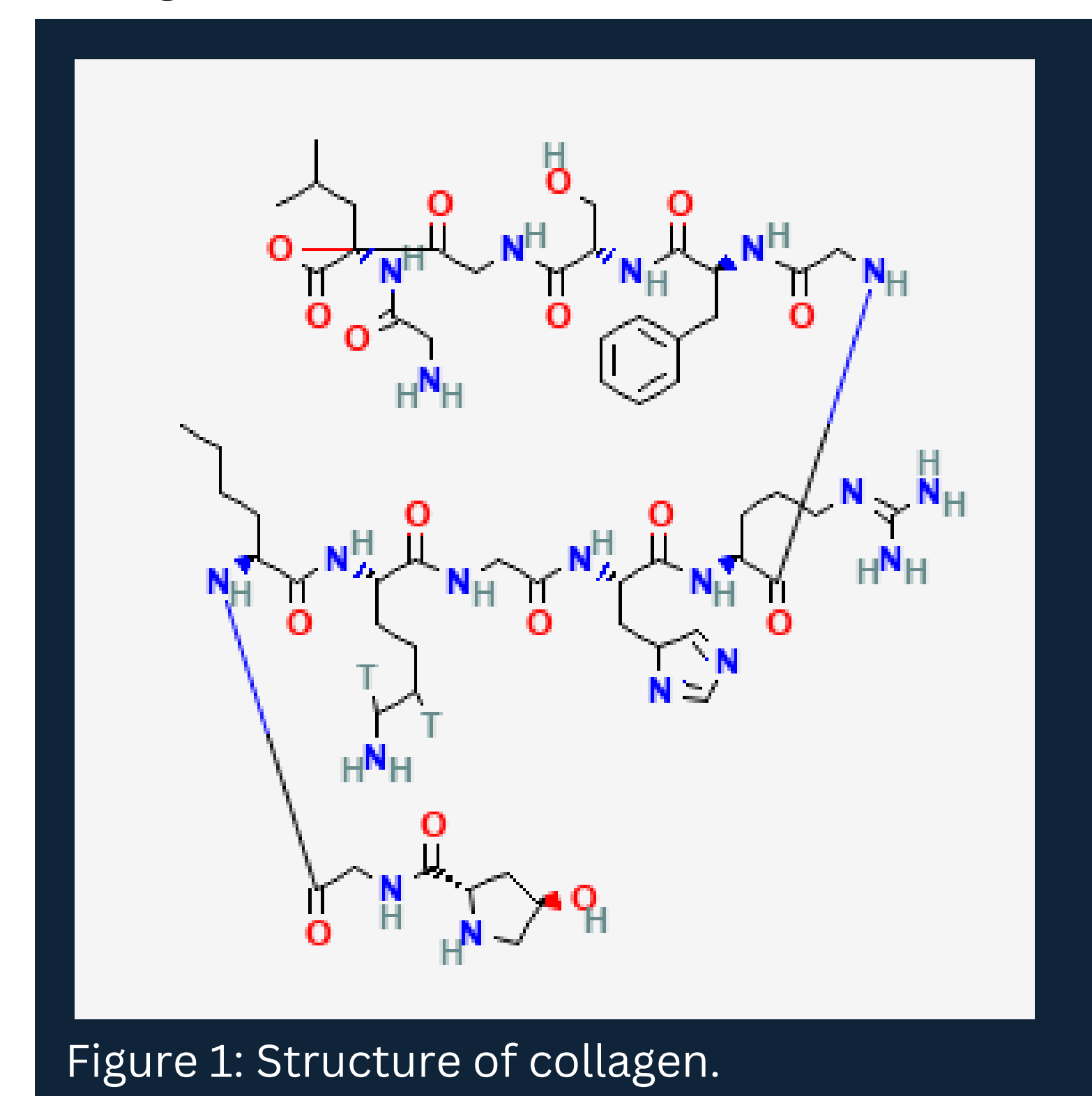


Figure 1: Structure of collagen.

Polymer Scaffold:

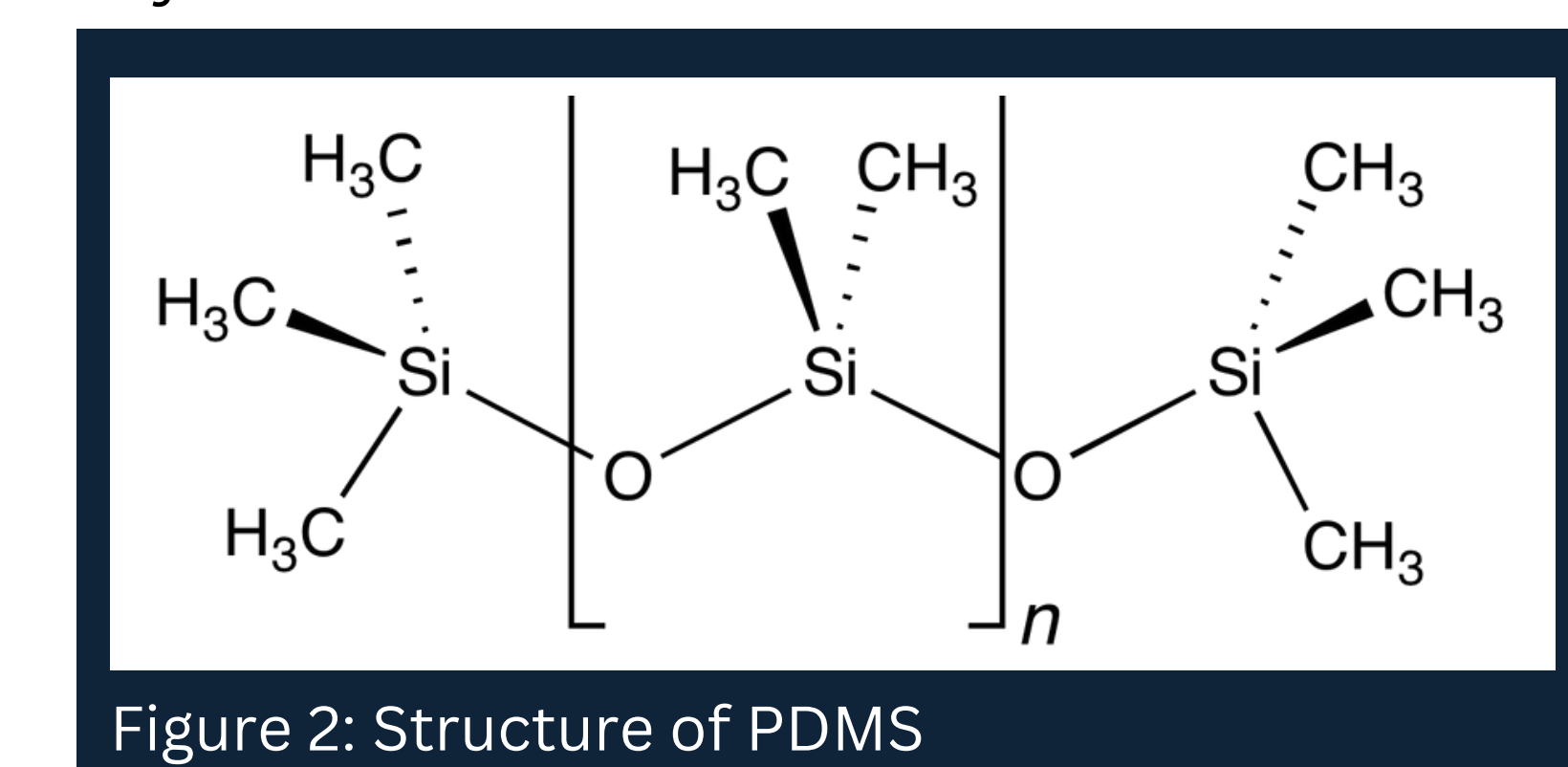


Figure 2: Structure of PDMS

- Collagen is a major component of the Extra cellular matrix (ECM); it is a favorable scaffold material for muscle tissue [1].
- Collagen has poor mechanical properties; it is sometimes combined with synthetic polymers such as poly-Lactic Acid (PLA) for structural support [2].
- Poly dimethyl siloxane (PDMS) has been used in scaffolds previously and has been noted for its flexibility and strong mechanical properties [3].
- We aim to create a scaffold using collagen and PDMS to facilitate C2C12 cell growth and ultimately make a more realistic tissue model [3]

Methods

Week 1

- After PDMS was prepared, it was placed into 6 wells, 0.5 mL/well.
- PDMS was crosslinked with collagen and placed into 3 wells, 0.5 mL/well.
- After setting the PDMS, 3 wells were plasma treated.
- C2C12 cells were seeded at a density of 1.9×10^4 cells/mL. 1 mL of cell solution was placed in each well.
- 1 day later, media with 10% FBS was removed and replaced with media with 2% FBS
- Images and cell counts were taken the following week.

Week 2

Based on the results from week 1, seen in Figure 3, the following procedure was made with the hypothesis to better the cell growth.

- Two 12 well plates were utilized for week 2, and in accordance with the results from week 1, the addition of a replica plate was made to be plasma treated.
- PDMS was made and placed into 4 rows, two rows of each well plate. PDMS was crosslinked with collagen and added to 1 row of each well plate.
- After the PDMS was completely set, two rows were cut with cross hatching, and a collagen mix was added to the cuts.
- A collagen gel was used as a control in 3 wells.
- Each well had 0.5 mL of its respective solution, excepting the 3 control wells with no solution.
- The plate without collagen was treated with plasma.

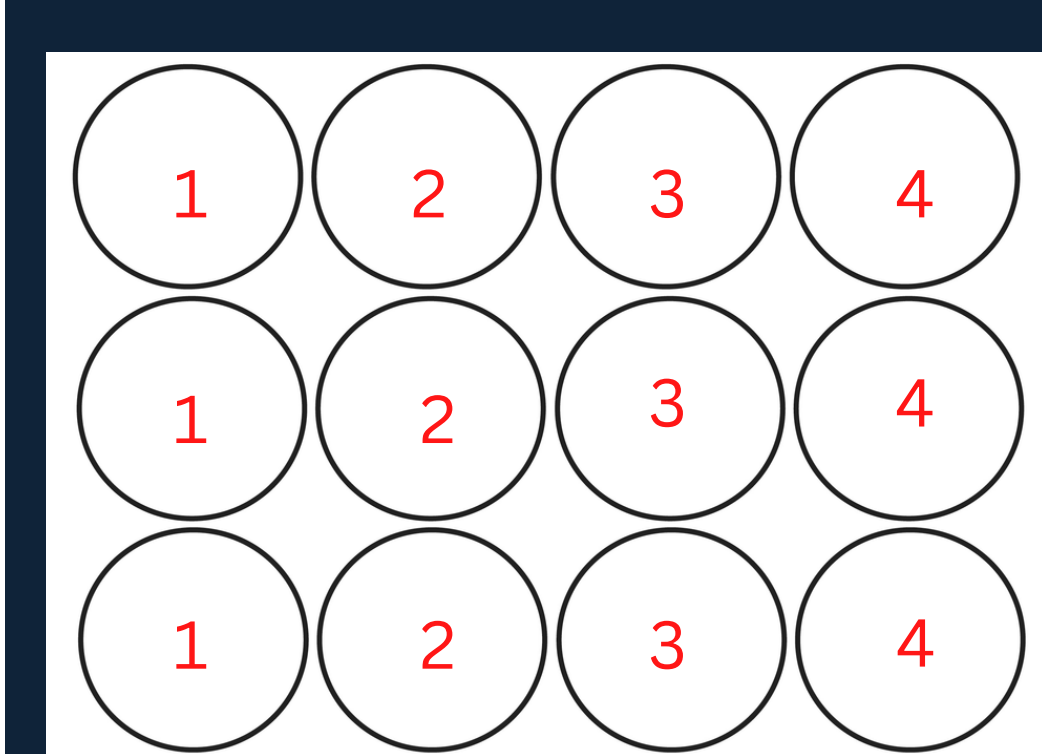


Figure 3: Week 1 well plate. Row 1 has plasma treated PDMS. Row 2 has non-plasma treated PDMS. Row 3 has PDMS crosslinked with collagen. Row 4 was the control without scaffold.

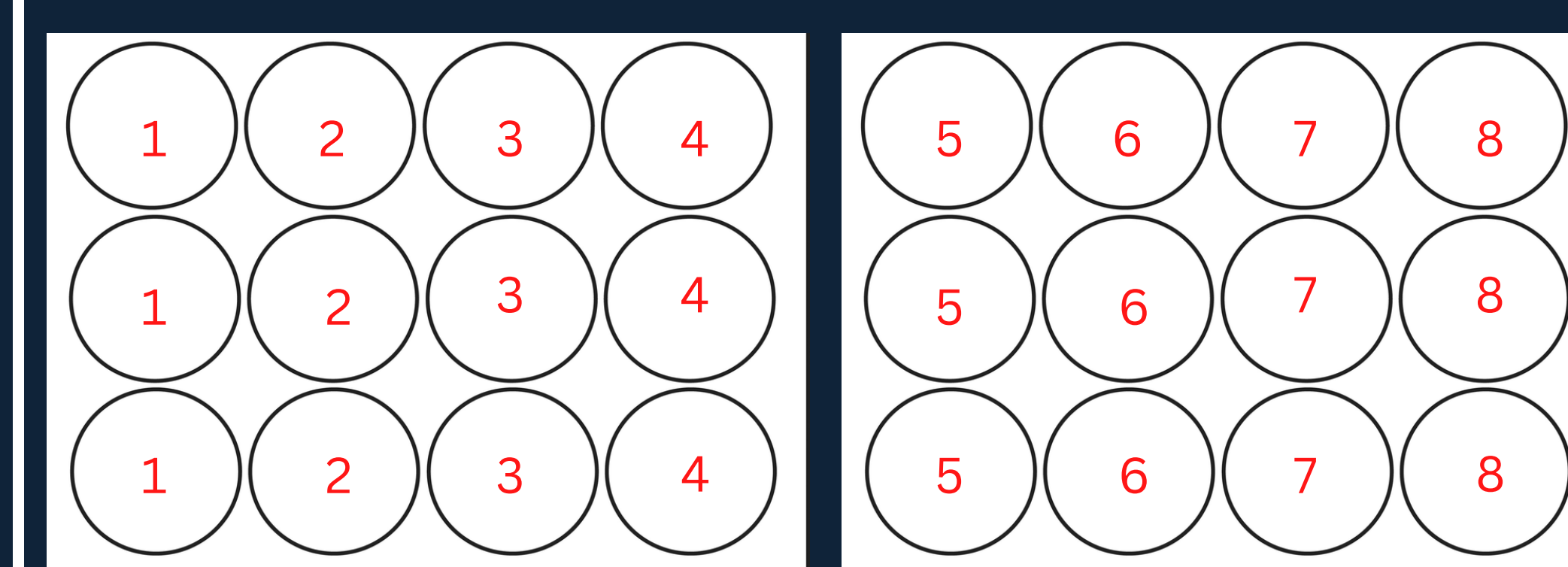


Figure 4: Week 2 well plates. Row 1 has plasma treated PDMS. Row 2 has PDMS crosslinked with collagen in the cross hatching. Row 3 has PDMS made with cross hatching with collagen in the cross hatching. Row 4 was the control without scaffold. Rows 1-3 were then plasma treated. Row 5 had non-treated PDMS. Row 6 had non-treated PDMS crosslinked with collagen. Row 7 has non-treated PDMS made with cross hatching with collagen in the cross hatching. Row eight has a collagen gel scaffold not plasma treated.

Results

From the first week of the experiment, we saw that the plasma treated PDMS scaffold had the highest cell growth in comparison with the control. In furthering the experiment to see if the PDMS can strengthen the mechanical properties of collagen, week 2 had a few more experiments to test collagen with PDMS in various structures.

In week 2's experiment, PDMS crosshatched with collagen gel and plasma treated was the most comparable to the control wells. Although this did not correspond with the hypothesis that the PDMS crosslinked with collagen and plasma treated would have the best growth.

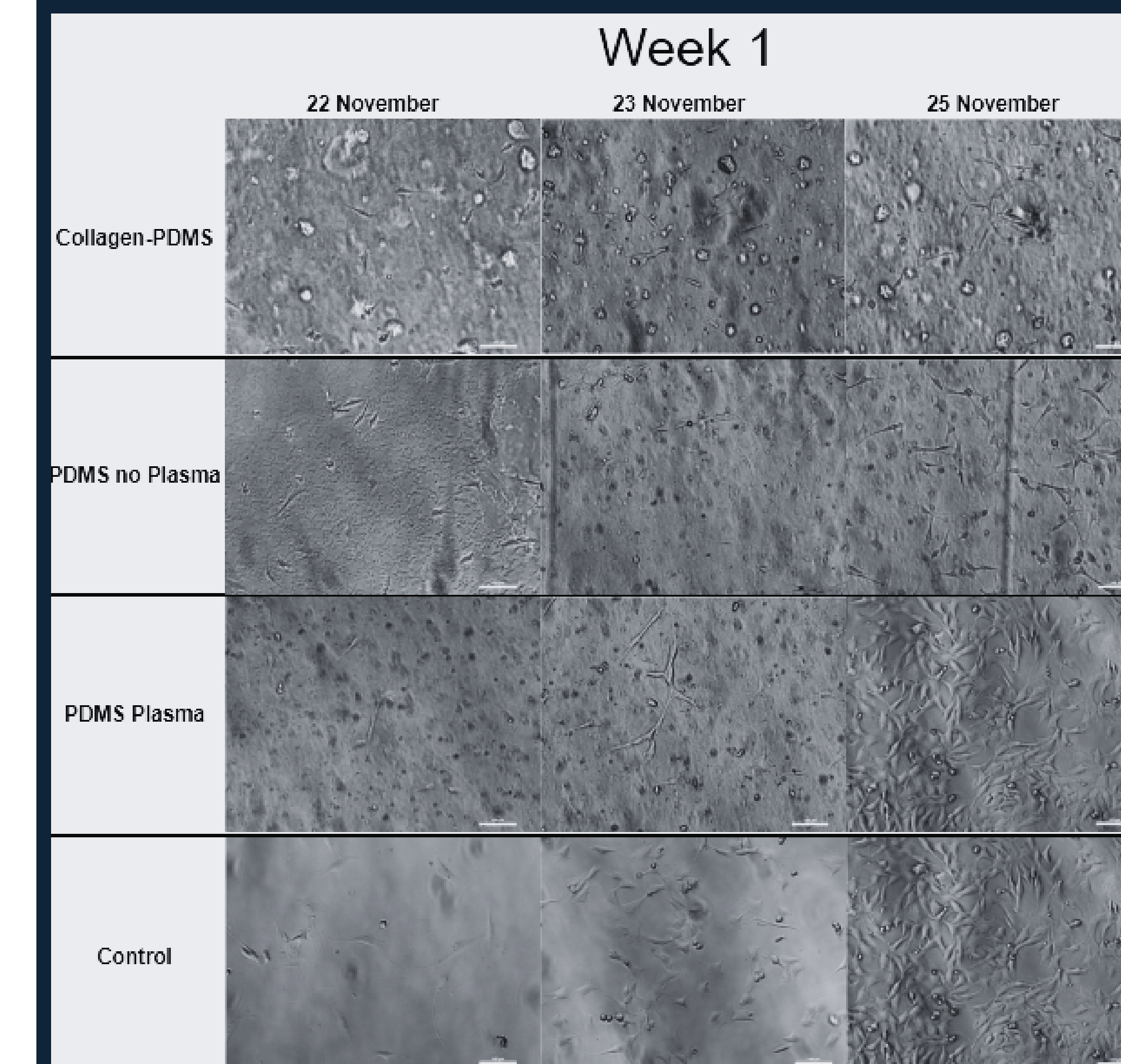


Figure 5: Images of the cells grown on their respective scaffold compared with the control well plate that had no scaffold material.

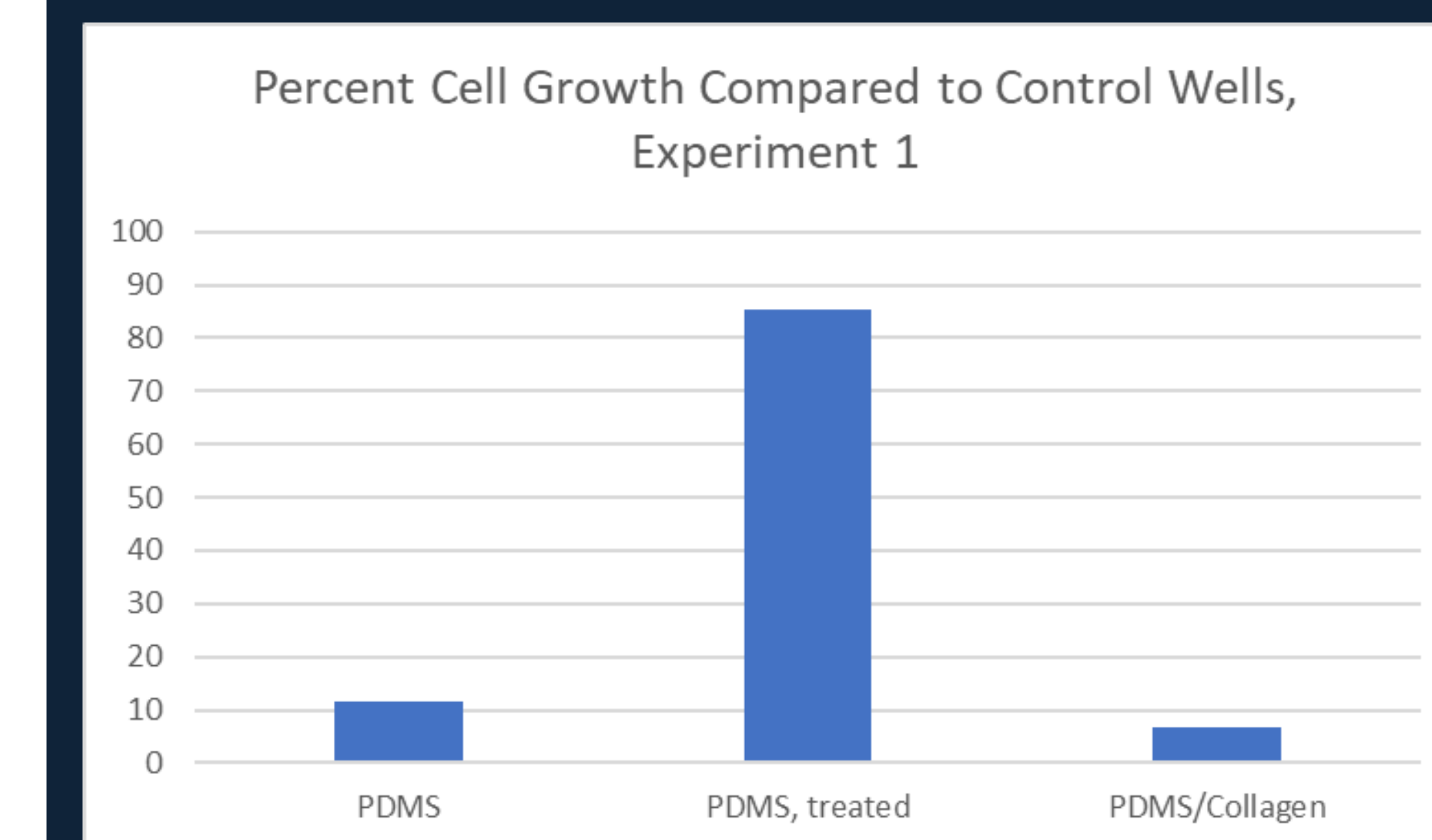


Figure 6: Cell viability of various materials compared to the viability of cells on a well plate with no additional materials.

Future Work

Moving forward, tensile tests should be performed with PDMS crosslinked with Collagen.

A repeat of this study should be conducted as results were not consistent for all material types.

After a repeat of the study has been completed, scaffolds of varying shapes should be created. This could help understand in what situations PDMS and Collagen would be best scaffold material.

References:

- [1] Jana, S.; Levensgood, S. K. L.; Zhang, M. Anisotropic Materials for Skeletal-Muscle-Tissue Engineering. *Adv. Mater.* **2016**, *28* (48), 10588–10612. <https://doi.org/10.1002/adma.201600240>.
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- [3] Varshney, N.; Sahi, A. K.; Vajanthri, K. Y.; Poddar, S.; Balavigneswaran, C. K.; Prabhakar, A.; Rao, V.; Mahto, S. K. Culturing Melanocytes and Fibroblasts within Three-Dimensional Macroporous PDMS Scaffolds: Towards Skin Dressing Material. *Cytotechnology* **2019**, *71* (1), 287–303. <https://doi.org/10.1007/s10616-018-0285-6>.

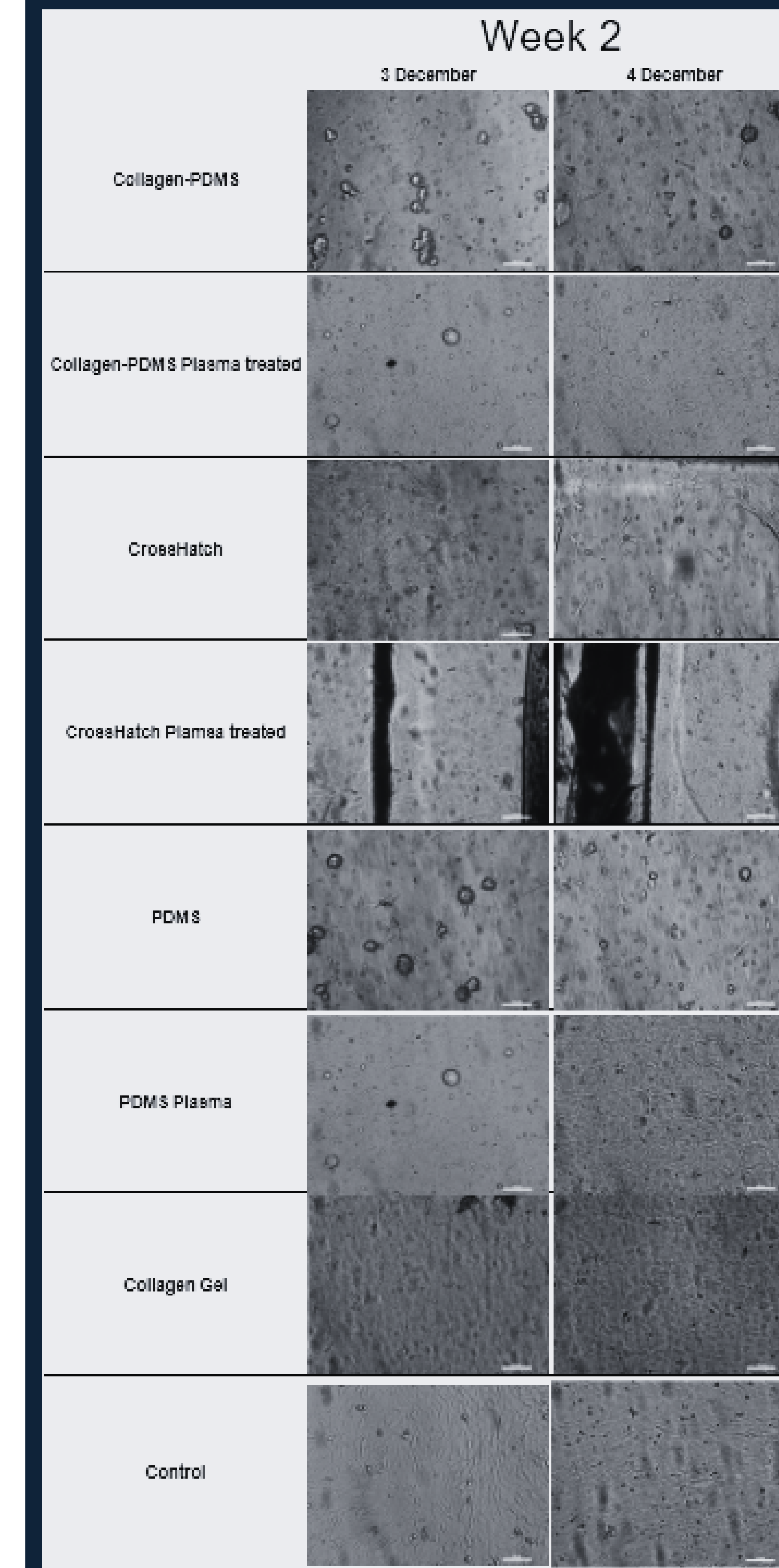


Figure 7: Images of the cells grown on their respective scaffold compared with the control well plate that had no scaffold material.

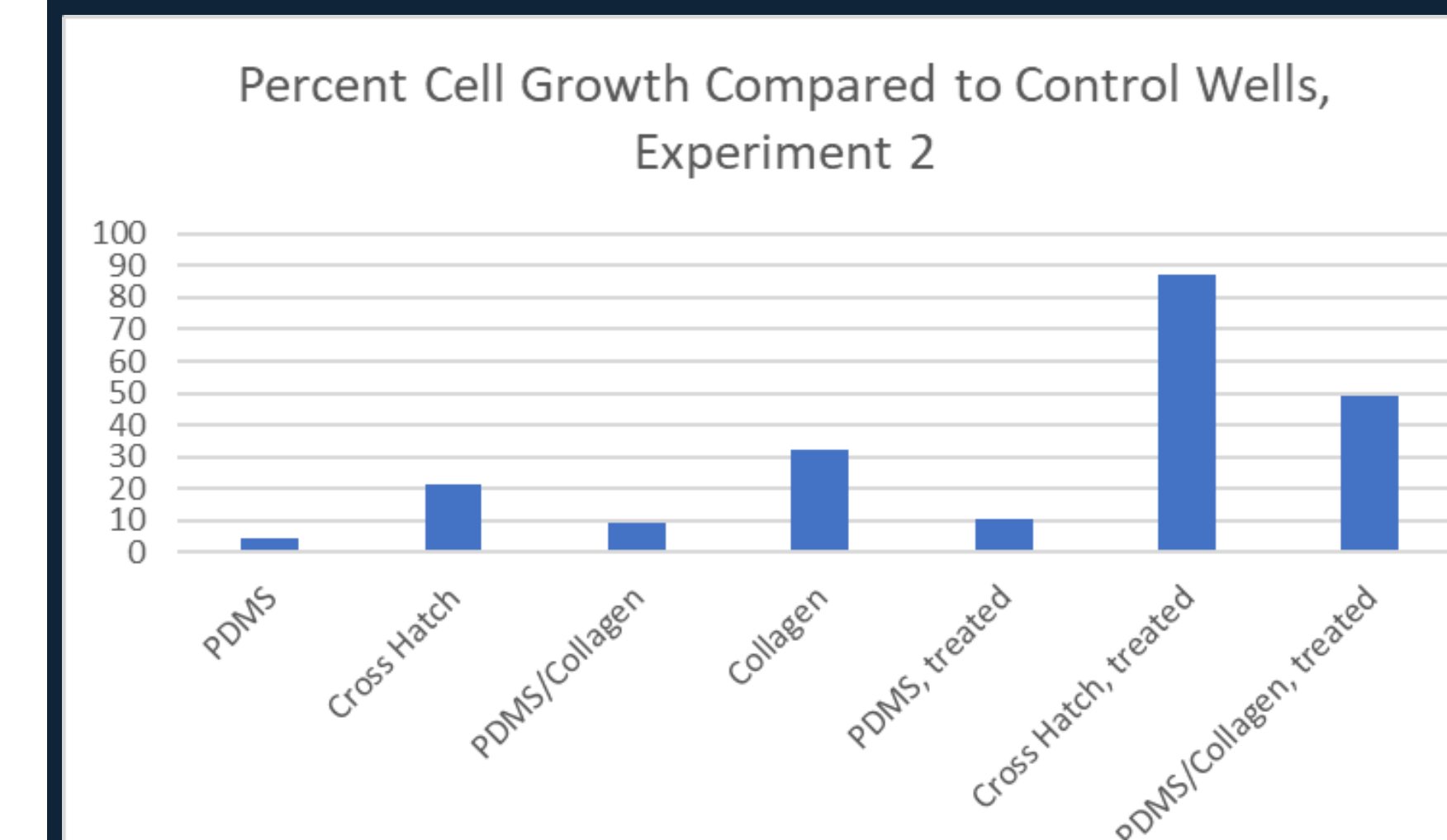


Figure 8: Cell viability of various materials compared to the viability of cells on a well plate with no additional materials