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Biomaterial Bilayers with Gelatin and Agar as Skin Substitutes

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ABSTRACT

This study evaluated five gelatin-, agar-, and bilayer-based biomaterial formulations as low-cost candidates for skin-substitute applications. Gelatin, agar, gelatin–silicon dioxide, agar–silicon dioxide, and gelatin–agar formulations were assessed using seven-day weight-loss measurements, three-point bending tests, and qualitative evaluations of flexibility and surface stability. The gelatin–agar bilayer demonstrated the lowest mean weight loss (7.3%) and maintained the greatest flexibility and surface stability, but exhibited the lowest mechanical strength. Agar-based formulations showed the highest resistance to bending but became increasingly rigid and brittle during storage. Statistical analyses indicated that the base material significantly affected peak load and relative flexural performance, whereas adding silicon dioxide did not significantly improve degradation resistance or mechanical performance. The gelatin–agar bilayer provided the most balanced physical performance, although further optimization is required to improve its mechanical strength. Future studies should standardize specimen dimensions and evaluate biocompatibility, physiological degradation, and wound-healing performance.

INTRODUCTION

Skin substitutes are a group of materials, biological, synthetic, or a mixture of the two, used to cover wounds where damage might have extended beyond the epidermal layer (Davison-Kotler et al., 2018). The invention was made in 1871, when Reverdin introduced skin grafting (a technique that moves healthy skin from a donor site to cover the patient's damaged skin). Even though skin grafting has limitations, including donor unavailability, immune reaction, pain, scarring, and slow healing infection, it was clinically useful. This technique was improved in 1880 by Joseph Gamgee, who used a cotton-and-gauze bilayer to cover the skin. In 1975, Rheinwald and Green cultured human skin cells on dead mouse skin that had been irradiated to explore the unique use of skin substitutes (Alrubaiy & Al-Rubaiy, 2009).

Skin substitutes are used in patients with skin injuries to support the regeneration of the epidermis, dermis, or both. In addition, it is gaining traction in the cosmetics and pharmaceutical industries, particularly as an alternative animal model for product testing. Recently, devices used in the biomedical field, such as cellular-level therapies like mesenchymal stem cell or growth factor delivery, and techniques like large-scale biofabrication, such as 3D printing, have improved their strategies in

developing novel biomaterials to replicate and capture the natural trait of the biological, architectural, and functional complexity of multilayer skin used for implementation (Yu et al., 2019).

In fact, the techniques for engineering and fabricating skin substitutes for wound healing have greatly improved over the years. New technology has advanced the development of artificial and natural skin substitutes. Skin substitutes can be created by synthesizing autologous (tissues, cells, and bone harvested from the patient's own body), allograft (tissues, cells, and bone harvested from the donor's body), xenogenic (tissues, cells, and bone harvested from a different species), or synthetic sources (man-made materials designed to mimic the natural tissues. Nevertheless, we still have not developed a fully functional skin substitute. It is still an ongoing process to produce a product that can fully replicate and vascularize like the native skin. In addition, the current skin substitute has limitations and does not meet patients' needs for greater user-friendliness, affordability, and longer-term viability (Vig et al., 2017).

Skin substitutes are used for temporary or permanent coverage of severe injuries or damaged skin. Various substitutes and biomaterials mimic the structure and function of healthy skin. Some provide support and protection, such as wound coverage, and assist in skin regeneration for conditions like severe burns or chronic wounds; others offer a greater barrier against bacterial infection, reduce pain from external stimulation, and help the body's natural healing process (Halim et al., 2010).

Various materials can be used for skin substitution, such as gelatin, agar, and their bilayers. These materials are hydrocolloids commonly used as gelling, thickening, or stabilizing agents in the food industry, differing in their sources, textures, and heat stability. Gelatin offers creamy textures, binding, and foaming for desserts, supplements, and photography. Agar provides firm, heat-resistant vegan gels for Asian jellies, bacterial cultures, and fiber supplements (Das et al., 2015).

Gelatin is produced by hydrolyzing collagen and has preserved many of collagen's important chemical properties. It's primarily composed of the amino acids glycerine, proline, and hydroxyproline. Compared with collagen, gelatin has lower antigenicity, making it less likely to trigger an immune response, and it's also more soluble in water and more cost-effective. Due to its high solubility, gelatin must be crosslinked with other materials or techniques to improve its mechanical stability before being used as a scaffold. Researchers have created and developed gelatin-based skin substitutes using techniques such as freeze-drying, electrospinning, and ultraviolet crosslinking (Sheikholeslam et al., 2018).

Gelatin scaffolds can be designed as single-layer or bilayer structures for dermal and epidermal regeneration. The process of adding cells such as fibroblasts, keratinocytes, or hair follicle stem cells can enhance wound healing by improving reepithelialization, vascularization, graft integration, and the formation of the dermal-epidermal junction. They can also be combined with other biomaterials such as hyaluronic acid and fibrinogen to improve their performance (Sheikholeslam et al., 2018).

Gelatin's biological properties closely resemble those of native skin, making it suitable for treating full-thickness wounds. However, unmodified gelatin has limited stability because it melts and degrades at

body temperature. To address this problem, researchers use different crosslinking methods to enhance stability, including the natural crosslinker genipin, the synthetic crosslinker EDC, a combination of both, and the physical dihydrothermal technique. The gelatin scaffolds exhibit high porosity, good wettability, and favorable mechanical properties. The improvement of the scaffold has resistance to enzymatic degradation, mechanical strength, and durability (Arif et al., 2020).

Agar is a highly hydrophilic polymer with excellent biocompatibility and unique gel-forming properties, making it a great material for tissue engineering. However, its moderate mechanical strength under wet conditions and slow degradation rate limit its use as a wound dressing on its own. To address this limitation, researchers have combined agar with type 1 collagen and fabricated composite scaffolds using either oven drying at 50 degrees Celsius or freeze-drying. Its mechanical testing showed that adding type-1 collagen reduced the stiffness of pure agar while increasing its toughness and flexibility. In addition, toxicity tests demonstrated that the crosslinked composites were not harmful to mice. These animal studies using rabbit skin wounds showed that the agar-collagen composites promote effective healing without infection, fluid leakage, or noticeable scar formation. These findings in the literature suggest that incorporating type-1 collagen improves the mechanical performance of agar and makes agar-collagen composite a great material for wound dressing (Bao et al., 2008).

However, there is a gap in what these studies have tried to find. Building on the literature, we aim to study bilayer materials for use as skin substitutes, using gelatin and agar, with a focus on cost-effectiveness. In this study, to test the behavior and properties of bilayer skin substitutes, we hypothesized that a bilayer skin substitute of silicone dioxide with gelatin will have higher tensile strength in a 3-point bend test than gelatin-only, agar-only, agar with gelatin, and agar with silicone dioxide due to greater stability in structure, moisture level, and degradation.

Additionally, a bilayer skin substitute of silicone dioxide with gelatin will lose less % mass than gelatin-only, agar-only, agar with SiO2, and gelatin with agar because of enhanced structural stability, reduced hydrophilicity, which reduces water absorption, and lower solubility when silicon dioxide is added.

METHODS

We tested five material combinations for skin substitutes. Table 1 shows the composition and ratios for synthesizing each material combination. Our first bilayer consists of silicon dioxide and gelatin, the second bilayer consists of agar and silicon dioxide, and the third bilayer consists of gelatin and agar.

Table 1

Material	Water (mL)	Gelatin (g)	Agar (g)	SiO_2 (g)
Gelatin	120	15	0	0
Gelatin + SiO_2 Bilayer	120	15	0	5
Agar	150	0	12	0
Agar + SiO_2 Bilayer	150	0	9	5
Gelatin + Agar Bilayer	120	10	10	0

For pure gelatin (120 ml distilled water, 15g gelatin) and agar (150 ml distilled water, 12g agar), we brought the water to a boil, added the gelatin or agar powder, then poured it into the petri dish and refrigerated it until it solidified. Bilayer constructs were fabricated by preparing gelatin solutions (15 g gelatin in 120 mL distilled water); bloomed for 5 min; heated in a warm water bath until fully dissolved; and then silicon dioxide was added to a final concentration of 5 g until completely dissolved. Then we cast the solution into a Petri dish, allowed it to sit for a few minutes until fully set, and refrigerated it for 2–4 hours until firm. We repeated the same step for agar solution (150 ml distilled water, 9g agar, 5 g SiO_2) and gelatin with agar solution (120 ml distilled water, 10g gelatin, and agar each). The agar-based groups (150 ml water and varying agar proportions) differ from the gelatin-based formulation (120 ml water) because agar does not solidify or thicken as much at the same water volume as the gelatin formulation

Final thickness was measured using a ruler. From each construct, 7.0 cm × 2.0 cm strips were cut for testing. Each condition was prepared in 3 replicates. This process was repeated with silicone as the first layer and gelatin or agar as the second. The thickness of each strip is not controlled, resulting in varying heights because heights aren’t measured as they're poured into the Petri dish.

Table 1.1: Dimensions of each strip for the weight loss test

Material	Length(cm)	Width (cm)	Thickness Range (cm)	n
Gelatin	7.0	2.0	0.3-0.5	3
Gelatin + SiO_2 Bilayer	7.0	2.0	0.4-0.5	3
Agar	7.0	2.0	0.4	3
Agar + SiO_2 Bilayer	7.0	2.0	0.4-0.7	3
Gelatin + Agar Bilayer	7.0	2.0	0.6	3

Table 2. Specimen dimensions (length × width × thickness) used in weight-loss testing, by group (mean thickness range): Gelatin 0.3–0.5 cm; Gelatin+SiO_2 0.4–0.5 cm; Agar 0.4 cm; Agar+SiO_2 0.4–0.7 cm; Gelatin+Agar 0.6 cm (full replicate-level data in Supplementary Table 2).

Mechanical performance was evaluated using 3-point bending with a support span until breaks were observed, recording the maximum force at failure, and tensile loading using incremental 200 g weights applied to a fixed gauge until the strip exhibited observable breaks, recording extension and failure load. We created a bracket using three sticks of length 10 cm each to hold the strip and two sticks standing vertically apart 1.3 cm. We performed the test on the scale and applied pressure until the strip broke. Specimen thickness was not standardized across formulation due to gelatin- and agar-based solution solidifying to different heights at equivalent pour volumes, and thincess was not measured prior to solidification. To account for this variability, we calculated a relative flexural metric for each specimen. This normalization allows flexural performance to be compared across specimens of differing geometry, although it does not fully substitute for testing under standardized dimensions. All 3-point bending tests were performed manually using man-made support sticks rather than an instrumented mechanical tester. As a result, reported peak load reflects discrete measurements taken at the point of visible failure rather than a continuous load-displacement curve, and finer-grained mechanical properties, such as elastic module or strain at failure, could not be calculated.

Stability and barrier performance were assessed by a degradation study in water at room temperature on days 0, 3, and 7, with mass loss (%) measured. Stability and barrier performance can be defined as the

material's ability to remain intact during degradation and to evaluate the material based on how well it maintains its physical integrity and protects against external influences. By observing the surface and testing the strips' flexibility, we interpret and evaluate them to determine their score. Stability was evaluated by measuring the material's degradation and mass loss over 7 days. We took each sample from the refrigerator, let it sit at room temperature for 15 minutes, and measured the mass of each strip on a scale. This test determines how long the skin substitute can maintain its structural integrity during use. This test was conducted because effective skin substitutes must remain structurally stable long enough to protect the wound, prevent contamination, and maintain a moist healing environment while new tissue regenerates.

The statistical test of weight loss and the 3-point bending test are both performed by calculating the mean, standard deviation (SD), and sample size (n) for each experimental group. A one-way analysis of variance (ANOVA) was performed to determine whether significant differences in weight loss or 3-point bending test results existed among five biomaterial groups: gelatin, a bilayer of gelatin and silicon dioxide, agar, a bilayer of agar and silicon dioxide, and a bilayer of gelatin and agar. Welch's independent-samples t-test was used to compare pairs of materials, including gelatin versus the bilayer of gelatin and silicon dioxide; agar versus the bilayer of agar and silicon dioxide; gelatin versus the bilayer of gelatin and agar; and agar versus the bilayer of gelatin and agar. Two-way ANOVA was used to evaluate the effects of base material (gelatin or agar), the addition of silicon dioxide, and their interaction. The gelatin-agar bilayer is excluded because it does not fit the experimental design of this test. The relative flexural metrics were calculated to account for differences among specimens and to reduce the effects of specimen size, allowing flexural performance to be compared across different formulations despite differing dimensions. All statistical analyses were performed using Python with SciPy, pandas, and Statsmodels libraries. Statistical significance was defined as $p < 0.05$.

RESULTS

Figure 1 and Table 2 showed that the gelatin-agar bilayer exhibited the best overall stability among all tested materials. It has the lowest average weight loss, at 7.3%, indicating greater resistance to degradation during the seven-day refrigeration observation period. In contrast, gelatin mixed with silicon dioxide exhibited the highest average weight loss of 24.6%, while pure gelatin had a weight loss of 22.3%. Among the gelatin-based formulations, the addition of silicon dioxide resulted in greater weight loss than in pure gelatin; this suggests that silicon dioxide did not improve degradation resistance under the conditions tested. Compared with the gelatin-based material, agar exhibited the second-worst stability, with an average weight loss of 25.9%. However, a bilayer of agar and silicon dioxide improves the original stability, with an average weight loss of 20.3%.

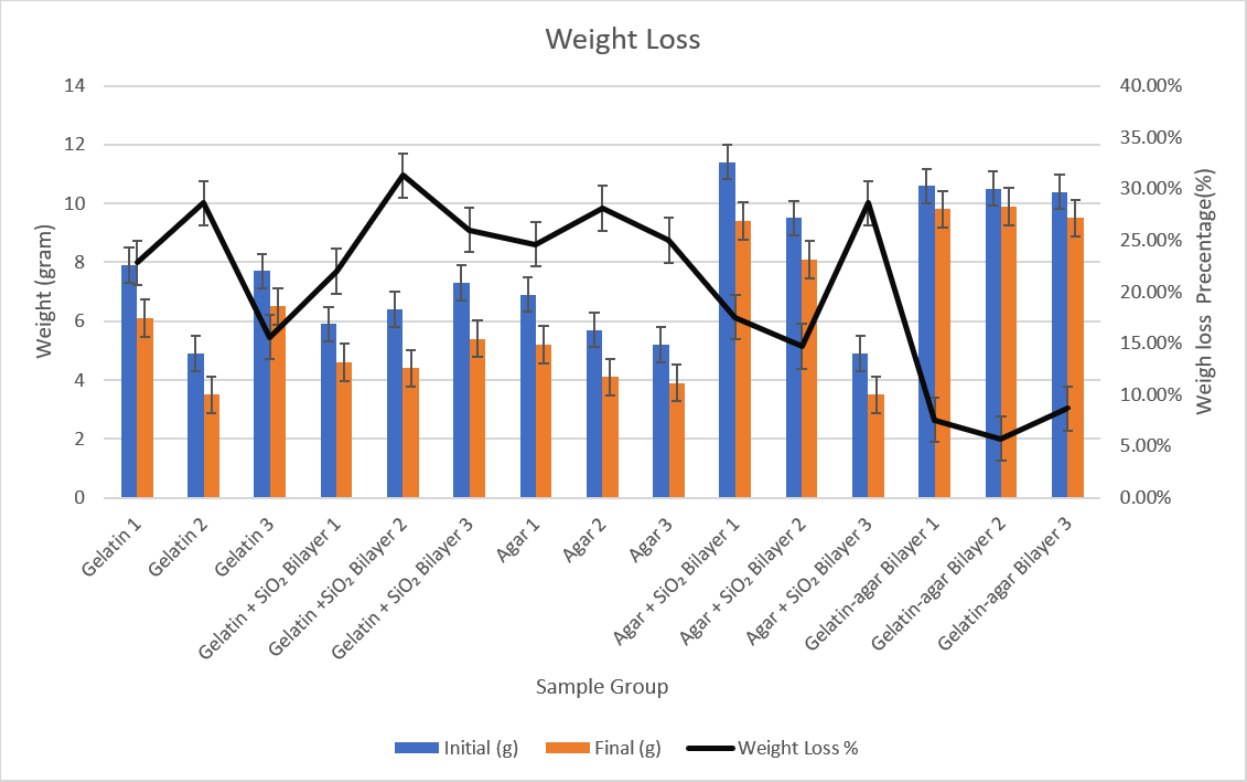

Figure 1. Weight loss of each sample. Comparison of initial weight, final weight, and total weight loss percentage across different biomaterial groups, including gelatin, agar, a bilayer that consists of silicon dioxide, and a bilayer of gelatin and agar.

Table 2: Average Weight Loss

Material Group	Average Weight Loss %	Sample size (n)
Gelatin	22.3%	3
Gelatin + SiO_2 Bilayer	26.4%	3
Agar	25.9%	3
Agar + SiO_2 Bilayer	20.3%	3
Gelatin + Agar Bilayer	7.3 %	3

Table 3 presents the results of the three-point bending test. Agar demonstrated the highest resistance to bending, indicating greater mechanical stiffness and structural strength. It performs better in the 3-point bending test, whereas the gelatin-agar bilayer exhibits the lowest ability to maintain structural stability under external forces. This reflects lower stiffness but greater flexibility. Pure gelatin and a bilayer of gelatin and silicon dioxide both exhibit similar strength and, in a 3-point bending test, yield higher structural strength than a bilayer consisting of gelatin and agar, but are more flexible than agar-based formulation samples.

Table 3: 3-point Bending Test

	Replicates		
Material	**1**	**2**	**3**
Gelatin	255g	287g	256g
Agar	815g	764g	538g
Bilayer gelatin + SiO_2	300 g	271g	160g
Bilayer agar + SiO_2	761 g	792 g	810 g
Bilayer gelatin + agar	147.7g	127.5g	125.1g

Flexibility was scored as 0 = fully flexible (baseline); 1 = slightly reduced flexibility; 2 = noticeable stiffness; 3 = mostly rigid, bends with difficulty; 4 = very rigid, cracks on bending; and 5 = brittle, breaks immediately. Both pure gelatin and the gelatin-silicon dioxide bilayer maintain high flexibility throughout the seven days of refrigeration, with only a slight decrease by Day 7. In comparison, pure agar and a bilayer of agar and silicon dioxide remain relatively rigid and become stiff and brittle over time. Agar-based samples exhibit the worst flexibility after 7 days of refrigeration. However, the gelatin-agar bilayer remains flexible for most of the observation period, showing only a slight reduction by Day 7.

The surface property is scored in the range of 0-5, as each score indicates 0 = smooth, dry, unchanged; 1 = slight roughness or mild tackiness; 2 = moderate tackiness / early erosion; 3 = clear surface degradation or stickiness; 4 = severe softening, slimy or highly eroded; and 5 = surface completely degraded / liquefying.

The table shows that gelatin and the gelatin-silicon dioxide bilayer exhibited only mild structural degradation, characterized by slight roughness and gradual moisture absorption over time. In contrast, pure agar and an agar bilayer with silicon dioxide showed more pronounced surface deterioration, progressing from a smooth surface to small, visible roughness and degradation by Day 7, indicating low resistance to prolonged storage. A gelatin-agar bilayer shows the best surface properties, with an initially smooth surface and slight roughness after 7 days of refrigeration.

Table 4 summarizes the mean weight loss, standard deviation (SD), and sample size for each biomaterial group. The gelatin-silicon dioxide bilayer has the highest mean weight loss and a less consistent sample group, with an SD of 4.62. Pure gelatin has a mean weight loss of 22.31% and high variability across sizes, with an SD of 6.51. Pure agar has an average weight loss of 25.9% and an SD of 1.89, indicating the samples are consistent. The bilayer of agar and silicon dioxide has an SD of 7.31, which is inconsistent across sample weights, and a mean weight loss of 20.28%. The gelatin-agar bilayer has an average weight loss of 7.31% and an SD of 1.48; it has the lowest SD among all formulations and exhibits less variation across samples.

Table 4: Statistical Test for Weight Loss

Group	Mean (%)	Standard Deviation	Sample Size (n)
Gelatin	22.3	6.5	3.0
Gelatin + SiO_2 Bilayer	26.4	4.6	3.0
Agar	25.9	1.9	3.0
Agar + SiO_2 Bilayer	20.3	7.3	3.0
Gelatin + Agar Bilayer	7.3	1.5	3.0

Table 5 presents the results of Welch's t-tests. The comparison between gelatin and the bilayer of gelatin and silicon dioxide revealed no statistically significant difference between the two groups, as indicated by a small t-value magnitude (-0.895) and a p-value (0.4265). This outcome suggests that the difference in mean weight loss between the two biomaterial groups was minimal relative to the variability within each group. Similarly, the comparison between agar and the agar bilayer with silicon dioxide also showed no statistically significant difference, as indicated by a p-value (0.3134) and a t-value (1.289). Likewise, the comparison between gelatin and the bilayer of gelatin and agar yielded a p-value(0.0511) and a t-value

(3.895). Although the t-value suggests a relatively large difference between group means, the p-value slightly exceeds 0.05. In contrast, the comparison between agar and the gelatin-agar bilayer produced a p-value of 0.0002 and a t-value of 13.421, indicating a statistically significant difference between the two groups. The large t-value demonstrates that the difference in mean weight loss between these formulation groups was much greater than the variability within the groups, confirming that these two materials differed in performance. It also presents the 95% confidence intervals. The comparison between agar and the gelatin-agar bilayer showed a 95% CI of [14.64, 22.56], which consists of the highly significant p-value. In contrast, the comparison between gelatin and gelatin-silicon dioxide bilayer showed a wider range of 95% CI.

Table 5: T-test for Weight Loss

Comparison	**Mean Difference (%)**	**95% CI**	**t-value**	**p-value**	**Significant (α = 0.05)**
Gelatin vs. Gelatin + SiO_2 Bilayer	4.12	[−17.44, 9.24]	-0.895	0.4265	No
Agar vs. Agar + SiO_2 Bilayer	-5.62	[−11.16, 22.36]	1.289	0.3134	No
Gelatin vs. Gelatin + Agar Bilayer	-15.01	[−0.14, 30.14]	3.895	0.0511	No
Agar vs. Gelatin + Agar Bilayer	-18.60	[14.64, 22.56]	13.421	0.0002	Yes

Table 6 presents the results of a two-way ANOVA comparing base materials (gelatin and agar), the formulation with silicon dioxide, and the interaction between base materials and silicon dioxide groups. This evaluates the effects of the base material, the addition of silicon dioxide, and their interaction on weight loss. The base material showed no significant difference (p = 0.6967), indicating that gelatin and agar did not differ significantly. Similarly, the addition of silicon dioxide formulations also has no significant effect, indicating no significant overall change in weight loss. In the comparison between the base material and silicon dioxide, the p-value is 0.1632, indicating no significant difference; this suggests that the effect of silicon dioxide on weight loss was consistent for both gelatin- and agar-based formulations.

Table 6: Two-way ANOVA test for Weight Loss

Source of Variation	Sum of Squares	df	F-value	p-value	Significant (α = 0.05)
Agar vs Gelain	4.931	1	0.163	0.6967	No
Agar + SiO_2 vs Gelatin + SiO_2	1.676	1	0.056	0.8196	No
Base Material × SiO_2	71.182	1	2.358	0.1632	No
Residual	241.458	8	—	—	—

Table 7 presents the peak load results for each biomaterial group, including the mean, standard deviation, and sample size. The agar-silicon dioxide bilayer exhibits the highest mean peak load (787.67) among the five biomaterial groups. Agar has the second-highest mean peak load (705.67) and the highest SD (147.43), indicating the greatest variability among samples. Compared with agar-based formulations, pure gelatin and a gelatin-silicon dioxide bilayer have lower peak load and SD. The gelatin-agar bilayer has the lowest mean peak load (133.43) and SD (12.41), indicating less variability within the sample group.

Table 7: Descriptive Statistics for Peak Load

Group	Mean (%)	Standard Deviation	Sample Size (n)
Agar	705.67	147.43	3
Agar + SiO_2 Bilayer	787.67	24.79	3
Gelatin + Agar Bilayer	133.43	12.41	3
Gelatin + SiO_2 Bilayer	243.67	73.89	3
Gelatin	266.00	18.19	3

Table 8 summarizes the results of descriptive statistics for the relative flexural metric of the five biomaterial formulations. Agar exhibits the highest mean relative flexural metric (255.93), indicating

superior flexural performance despite its dimensional differences, followed by the agar-silicon dioxide bilayer (229.64). In contrast, the gelatin-agar bilayer showed the lowest mean value (76.25), while the gelatin-silicon (90.10) and gelatin (101.34) bilayers showed intermediate values. The bilayer of gelatin and silicon dioxide group had the greatest variability (SD=45.80), followed by agar (SD=45.22), whereas the bilayer of gelatin and agar exhibited the smallest variability (SD=7.09), indicating more consistent measurements.

Table 8: Descriptive Statistics for Relative Flexural Metric

Group	Mean (%)	Standard Deviation	Sample Size (n)
Agar	255.93	45.22	3
Agar + SiO_2 Bilayer	229.64	7.23	3
Gelatin + Agar Bilayer	76.25	7.09	3
Gelatin + SiO_2 Bilayer	90.10	45.80	3
Gelatin	101.34	38.69	3

Table 9 presents the results of a two-way ANOVA comparing base materials (gelatin and agar), the formulation with silicon dioxide, and the interaction between base materials and silicon dioxide groups. This evaluates the effects of the base material, the addition of silicon dioxide, and their interaction on peak load. The base material(gelatin and agar) showed a significant difference ($p = 0.000008$), indicating that gelatin and agar differ significantly in their mean peak load. In contrast, the addition of silicon dioxide formulations has no significant effect, indicating that the addition of silicon dioxide did not significantly affect peak load under the conditions of this study. In the comparison between the base material and silicon dioxide, the p-value is 0.312772, indicating no significant difference and suggesting that the effect of silicon dioxide on peak load was similar for both gelatin- and agar-based silicon formulations.

Table 9: Two-Way ANOVA for Peak Load

Source of Variation	F-value	p-value	Significant?
Agar vs Gelatin	103.156	0.000008	Yes
Agar + SiO_2 vs Gelatin + SiO_2	0.380	0.554968	No

Base Material × SiO_2	1.160	0.312772	No

Table 10 presents the results of the two-way ANOVA examining the effects of the base material, the addition of silicon dioxide, and their interaction on the relative flexural metric. The analysis showed that the base material had a statistically significant effect on the relative flexural metric (p-value = 0.000145), indicating that gelatin and agar-based formulations differed significantly in their mean relative flexural metrics. In contrast, the addition of silicon dioxide did not have a statistically significant effect on the relative flexural metric (p-value = 0.414058), suggesting that incorporating silicon dioxide did not significantly alter the relative flexural metric under the conditions of this study. Furthermore, no significant interaction was observed between the base material and silicon dioxide (p-value = 0.738631), indicating that the effect of silicon dioxide on the relative flexural metric was consistent for both gelatin and agar-based formulations.

Table 10: Two-Way ANOVA for Relative Flexural Metric

Source of Variation	F-value	p-value	Significant?
Agar vs Gelain	45.597	0.000145	Yes
Agar + SiO_2 vs Gelatin + SiO_2	0.742	0.414058	No
Base Material × SiO_2	0.119	0.738631	No

Table 11 presents the results of Welch's t-test for the peak load mean. The comparison between gelatin and the bilayer of gelatin and silicon dioxide revealed no statistically significant difference between the two groups, as indicated by a small t-value (0.508) and a p-value (0.6569). This result suggests that the mean peak loads of the two biomaterial groups were similar, and any observed differences were likely due to variation among samples rather than between formulations. Similarly, the comparison between agar and the agar bilayer with silicon dioxide also showed no statistically significant difference, as indicated by a p-value of 0.4377 and a t-value of -0.950, indicating that adding silicon dioxide did not significantly affect the mean peak load. In contrast, the comparison between gelatin and the gelatin-agar bilayer yielded a p-value of 0.0009 and a t-value of 10.425. In addition, the comparison between agar and the gelatin-agar bilayer produced a p-value of 0.0208 and a t-value of 6.699, indicating a statistically significant difference between the two groups. The larger t-value demonstrates that the difference in mean peak load between these formulation groups was much greater than the variability within the groups, confirming that these two materials differed in performance. It also presents 95% confidence intervals. The comparison of gelatin and gelatin-agar bilayer and the comparison of agar and gelatin agar bilayer

has a range that excludes zero. While the comparison of gelatin and gelatin-silicon dioxide bilayer and the comparison of the agar and agar-silicon dioxide bilayer had wider intervals that included zero.

Table 11: Welch's t-Test Results of peak load

Comparison	Mean Difference (g)	95% CI	t-value	p-value	Significant?
Gelatin vs Gelatin + SiO_2 Bilayer	-22.33	[−148.47, 193.13]	0.508	0.6569	No
Agar vs Agar + SiO_2 Bilayer	82.00	[−434.98, 270.98]	-0.950	0.4377	No
Gelatin vs Gelatin + Agar Bilayer	-132.57	[95.34, 169.80]	10.425	0.0009	Yes
Agar vs Gelatin + Agar Bilayer	-572.23	[209.59, 934.89]	6.699	0.0208	Yes

DISCUSSION

The purpose of this study was to evaluate the mechanical performance, degradation resistance, flexibility, and surface stability of gelatin, agar, and bilayer-based biomaterials for potential use as skin substitutes. We predicted that gelatin mixed with silicon dioxide would have the highest mechanical strength and the lowest degradation due to improved structural stability and reduced water absorption. However, the results only partially supported the hypothesis. The addition of silicon dioxide slightly improved agar's degradation resistance but worsened gelatin's performance. The gelatin-agar bilayer showed the best overall stability, with the lowest average weight loss, greater flexibility, and greatest surface stability during seven days of refrigeration. Nevertheless, it performed poorly in 3-point bending tests, indicating reduced mechanical strength and low capacity to withstand external loading. These findings describe the physical characterization of candidate biomaterials only and no biological validation was performed in this study. Suitability as a functional skin substitute additionally requires evaluation of cytocompatibility, cell adhesion and proliferation, antimicrobial performance, swelling behavior, and wound-healing capacity in vivo or in relevant cell-based models. The gelatin and agar bilayer’s favorable physical profile makes it a reasonable candidate for such follow-up biological testing, but its viability as a skin substitute cannot be established from mechanical and degradation data alone.

The weight-loss analysis showed that the gelatin-agar bilayer had the greatest resistance to degradation during refrigerated storage. This formulation had the lowest average weight loss and the smallest standard deviation, suggesting better moisture retention and consistent performance across samples. The

combination of gelatin and agar reduces moisture migration and slows degradation, improving preservation throughout storage. In contrast, adding silicon dioxide did not significantly improve degradation resistance in gelatin or agar formulations. The gelatin-silicon dioxide bilayer had slightly greater weight loss than pure gelatin, while the agar-silicon dioxide bilayer showed a modest improvement over pure agar. Two-way ANOVA found no significant effect of silicon dioxide on weight loss. Welch's t-test also found no significant difference between base materials and those with silicon dioxide. These findings indicate that silicon dioxide, at the concentration and formulation used, did not significantly improve the storage stability of either biomaterial.

Mechanical analysis showed a different result. Agar had the highest bending resistance, with a greater peak load and higher relative flexural metric than gelatin-based formulations. In comparison, the gelatin-based formulation had a lower peak load but greater flexibility because gelatin forms a softer protein network that can undergo large elastic deformation before failure. Although agar had the strongest mechanical strength, it showed the poorest flexibility and surface stability during refrigerated storage. This was mainly due to agar's rigid network compared to gelatin's elastic structure, leading to different performance. The flexibility score indicated that agar-based formulations became stiffer and more brittle over time. Surface evaluation showed gradual roughness and degradation after seven days. These changes likely result from moisture loss during storage, which increases rigidity and reduces surface integrity. In contrast, the gelatin and silicon dioxide bilayer retained high flexibility throughout the observation period, with only a minor reduction by Day 7. The gelatin-agar bilayer also maintained good flexibility despite the presence of agar, suggesting that gelatin helps make up agar's rigidity. Surface observations support the weight-loss degradation results. The gelatin-agar bilayer maintained the smoothest surface during storage, while agar-based formulations showed earlier signs of roughening and erosion. A gelatin-agar bilayer exhibits superior surface characteristics, suggesting that combining gelatin and agar enhances resistance to moisture-related deterioration.

Statistical analysis showed that the base material was the primary factor influencing both mechanical and degradation performance. Two-way ANOVA revealed that the type of base material significantly affected peak load and the relative flexural metric, whereas the addition of silicon dioxide did not have a significant effect. There was no significant interaction between base material and silicon dioxide, indicating silicon dioxide's effect was similar whether combined with gelatin or agar. Welch's t-test also found no significant difference between pure materials and those with silicon dioxide. Therefore, under the conditions of this study, silicon dioxide did not provide measurable improvements in degradation resistance or mechanical performance.

Several limitations should be considered when interpreting these findings. The study evaluated biomaterials only under refrigerated storage for seven days and focused on physical and mechanical properties. It is important to note that degradation was assessed under refrigerated storage over seven days, conditions that differ substantially from the physiological environment a skin substitute would encounter in clinical use, including body temperature(37 degree celsius), a buffered ionic environment, exposure to wound exudate and enzymatic activity, and continuous mechanical loading. The weight-loss

and stability results reported therefore reflect storage stability under laboratory conditions rather than in vivo degradation behavior, and should not be interpreted as predictive of clinical performance. Future studies should evaluate degradation under simulated physiological conditions, such as phosphate-buggered saline or cell culture media at 37 degree celsius with enzymatic challenge, to better approximate the wound environment. The thickness of each strip across the five biomaterial groups was inconsistent, resulting in large standard deviations within groups. Due to specimen geometry not standardized and testing was performed manually rather than with an instrumented device, raw peak load values presented in table 7 should be interpreted cautiously. The relative flexural metric shown in table 8, which helps to normalize for specimen size, is a more reliable basis for comparing formulations. Future work should adopt standardized specimen dimensions and instrument-based testing to enable direct comparison with border biomaterial literature. Silicon dioxide was added in powder form rather than as a liquid at a specific concentration. Surface properties and flexibility were assessed by observation rather than specific formulas, which may lead to biased conclusions. The three-point bending test is performed manually, without any specific machine. Other important characteristics, including swelling behavior, tensile strength, biodegradation under physiological conditions, antimicrobial activity, and long-term stability, were not investigated. A further limitation of this study is the small sample size(n = 3 in each formulation group) There are only three replicates per condition. The statistical power to detect the true difference between formulations is limited. Statistically significant comparison based on n=3 should be interpreted with some caution, as small samples are more susceptible to the influence of individual outlier measurements and may not generalize reliably to a larger population of specimens. Future studies should increase the number of replicates per formulation to ideally n >= 6-8 to improve statistical power and reliability of comparisons. Several non-significant comparisons had wide confidence intervals spanning zero, suggesting the study may be underpowered to detect a true difference rather than confirming its absence

These findings indicate that the gelatin-agar bilayer offers the most favorable combination of degradation resistance, flexibility, and surface stability, making it the best-performing formulation among those evaluated. Although agar exhibited superior mechanical strength, its increased rigidity and reduced storage stability limit its ability. The addition of silicon dioxide did not significantly improve either degradation resistance or mechanical properties under the experimental conditions. Future studies should investigate different silicon dioxide concentrations, alternative reinforcement materials, and longer storage periods to further optimize the performance of biomedical applications.

CONCLUSION

This study aimed to evaluate the mechanical properties, degradation resistance, flexibility, and surface stability of gelatin-, agar-, and bilayer-based biomaterials. We hypothesized that the gelatin-silicon dioxide bilayer will be a promising candidate for a cost-effective skin substitute. By performing a weight-loss test, a 3-point bending test, a surface properties test, a flexibility test, and statistical analysis, we can determine which biomaterial group is best suited in terms of cost-effectiveness and its ability to

serve as a skin substitute. However, the result does not support the original hypothesis. The results demonstrated that each formulation exhibits distinct advantages and limitations. The agar-based formulation exhibits greater resistance and a higher capacity to withstand external forces, whereas the gelatin-based formulation has greater flexibility during the refrigerated period. The gelatin-silicon dioxide bilayer exhibited modest results in each test performed throughout the seven-day refrigeration period, with flexibility and surface properties similar to those of pure gelatin, but reduced resistance to deterioration. These results suggest that the addition of silicon dioxide did not significantly improve performance in either gelatin or agar, indicating that the difference is due to the base material used in the formulation.

In all groups, the gelatin-agar bilayer has the most balanced overall performance; it exhibited the lowest mean weight loss, maintained flexibility throughout the storage period, and showed the greatest surface stability, indicating enhanced resistance to degradation. However, it performs the worst in mechanical strength on the three-point bending test, indicating the lowest capacity to bear external forces. Further research will need to investigate in greater detail the effects of varying silicon dioxide concentrations across formulations to determine their impact on biomaterials and skin substitutes.

COMPETING INTERESTS

There were no competing interests.

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DECLARATION

Artificial intelligence (ChatGPT by OpenAI) was used to assist with Python code in this study. All code was reviewed, tested, and modified by the author before implementation. The experimental setup, data collection, analysis, and interpretation of results were completed independently.

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APPENDIX

Supplementary Table 1: Weight loss of each sample

Materials	**Initial (g)**	**Final (g)**	**Weight Loss %**
Gelatin 1	7.9	6.1	22.8%
Gelatin 2	4.9	3.5	28.6%
Gelatin 3	7.7	6.5	15.6%
Gelatin + SiO_2 Bilayer 1	5.9	4.6	22.0%
Gelatin +SiO_2 Bilayer 2	6.4	4.4	31.3%
Gelatin + SiO_2 Bilayer 3	7.3	5.4	26.0%
Agar 1	6.9	5.2	24.6%
Agar 2	5.6	4.1	28.1%
Agar 3	5.2	3.9	25.0%
Agar + SiO_2 Bilayer 1	11.4	9.4	17.5%
Agar + SiO_2 Bilayer 2	9.5	8.1	14.7%
Agar + SiO_2 Bilayer 3	4.9	3.5	28.6%
Gelatin-agar Bilayer 1	10.6	9.8	7.55%

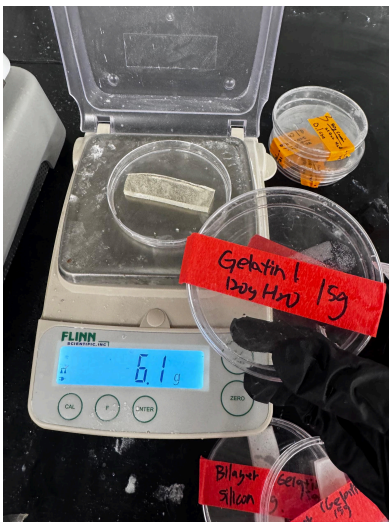
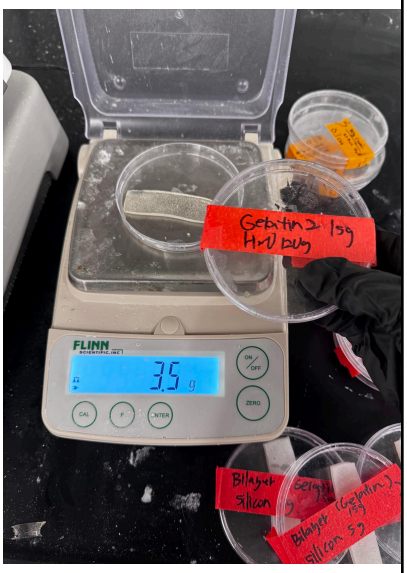
Gelatin-agar Bilayer 2	10.5	9.9	5.71%
Gelatin-agar Bilayer 3	10.4	9.5	8.65%

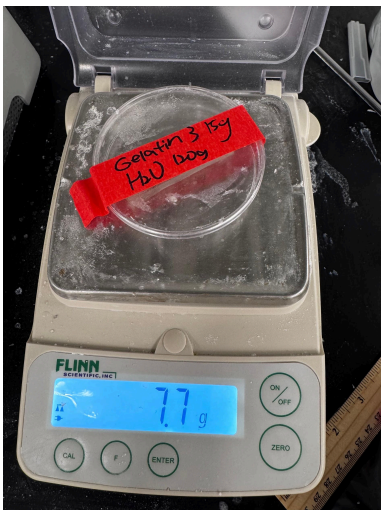
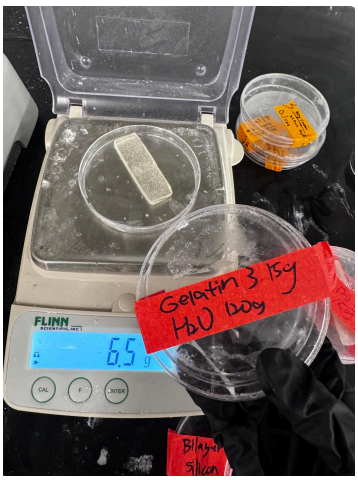
Supplementary Table 2: Dimensions of each strip for the weight loss test

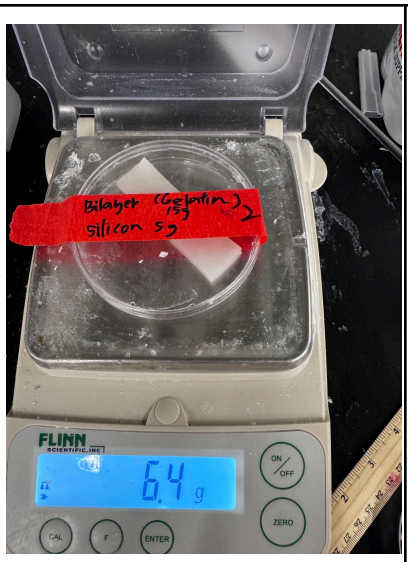
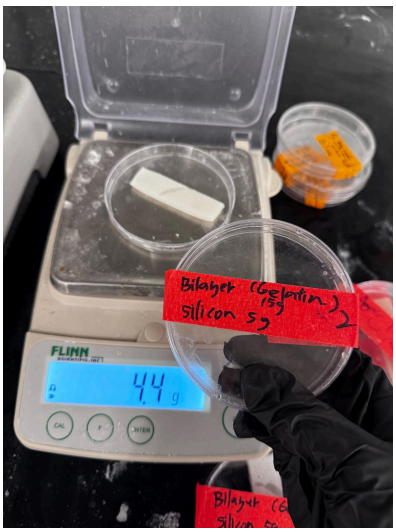
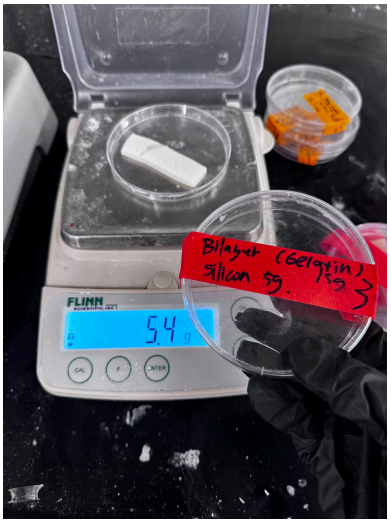
Material	**Sample 1**	**Sample 2**	**Sample 3**
Gelatin	7.0 × 2.0 × 0.5 cm	7.0 × 2.0 × 0.3 cm	7.0 × 2.0 × 0.5 cm
Gelatin + SiO_2 Bilayer	7.0 × 2.0 × 0.4 cm	7.0 × 2.0 × 0.5 cm	7.0 × 2.0 × 0.5 cm
Agar	7.0 × 2.0 × 0.4 cm	7.0 × 2.0 × 0.4 cm	7.0 × 2.0 × 0.4 cm
Agar + SiO_2 Bilayer	7.0 × 2.0 × 0.7 cm	7.0 × 2.0 × 0.7 cm	7.0 × 2.0 × 0.4 cm
Gelatin + Agar Bilayer	7.0 × 2.0 × 0.6 cm	7.0 × 2.0 × 0.6 cm	7.0 × 2.0 × 0.6 cm

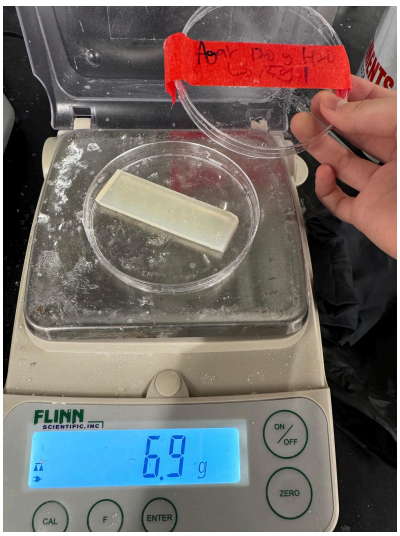
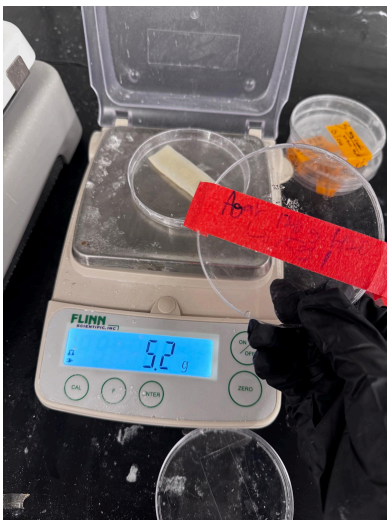
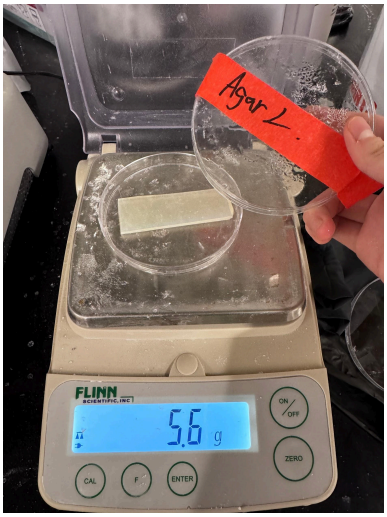
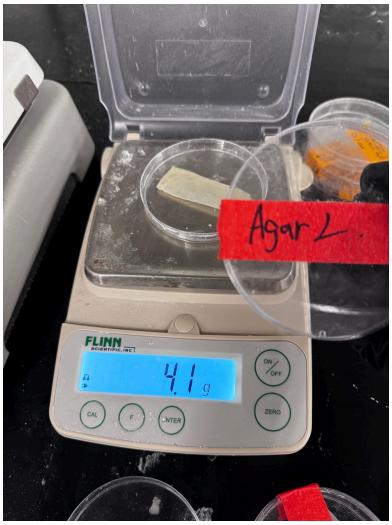
Supplementary Table 3: Photography of Each Biomaterials

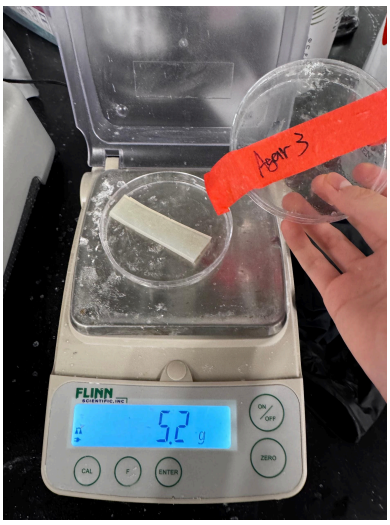
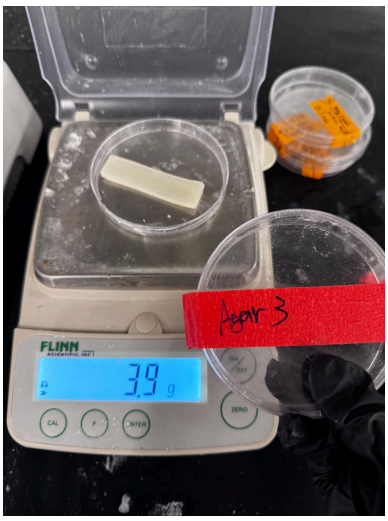
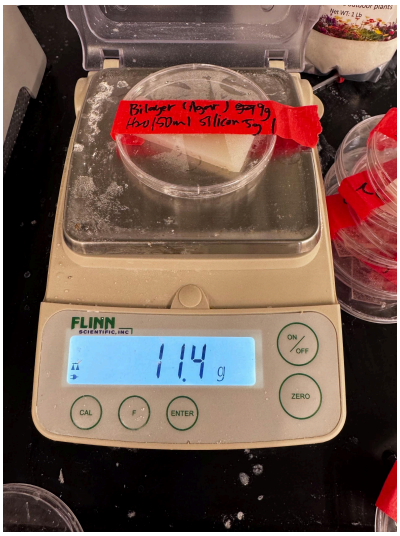
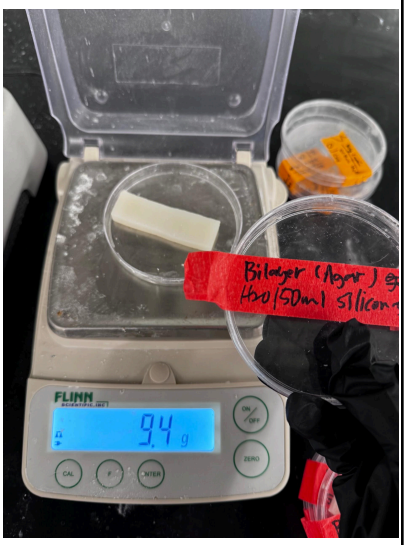
Biomaterial Group	Day 0	Day 7

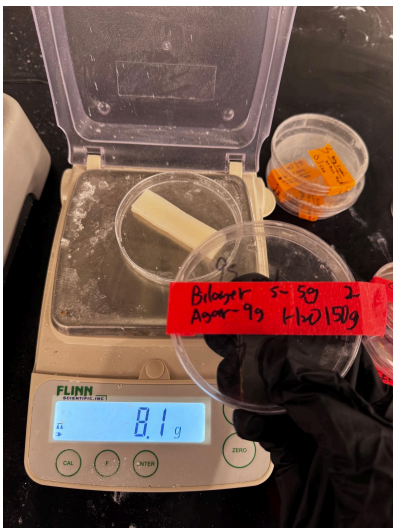
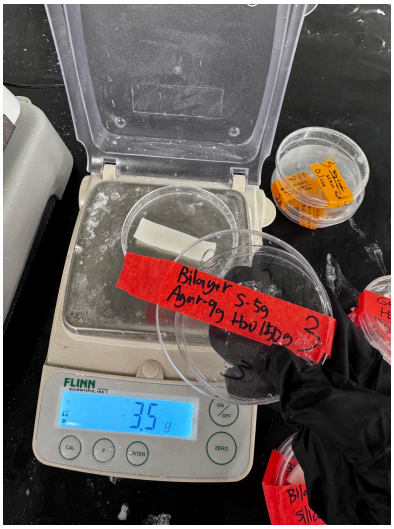
Gelatin 1		
Gelatin 2		

Gelatin 3		
Gelatin + SiO_2 Bilayer 1		

Gelatin +SiO_2 Bilayer 2		
Gelatin + SiO_2 Bilayer 3		

Agar 1	 FLINN SCIENTIFIC, INC 6.9 g ON/OFF ZERO CAL ENTER	 FLINN 5.2 g
Agar 2	 Agar 2. FLINN SCIENTIFIC, INC 5.6 g ON/OFF ZERO CAL ENTER	 Agar 2. FLINN 4.1 g

Agar 3		
Agar + SiO_2 Bilayer 1		

Agar + SiO_2 Bilayer 2		
Agar + SiO_2 Bilayer 3		

Gelatin-agar Bilayer 1