

***In vitro* evaluation of the tensile strength and degradation of two types of collagen membranes: Geistlich Bio-Gide and Tisum Sus-Mem**

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Abstract

Following tooth extraction, bone resorption and remodeling are inevitable; therefore, guided bone regeneration (GBR) is essential in current implantology. In this procedure, collagen membranes act as biological barriers that prevent epithelial ingrowth and promote osteogenesis. The objective of this study was to compare *in vitro* the tensile strength and degradation of the Geistlich Bio-Gide and Tisum Sus-Mem collagen membranes. The research methodology was experimental, comparative, preclinical, and *in vitro*. The study population and sample comprised 68 specimens, which were distributed among analyses of surface morphology, mechanical properties, hydrophilicity, and degradation. Chemical composition and morphology were analyzed by means of scanning electron microscopy and EDS analysis; hydrophilicity was determined through contact-angle measurements; mechanical properties were examined by uniaxial tensile tests under wet and dry conditions; and, finally, degradation was investigated in a collagen solution and in artificial saliva over 7, 14, and 21 days. The findings revealed functional and structural differences between the two membranes. Bio-Gide showed much higher hydrophilicity ($p < 0.001$) and had a well-defined bilayer morphology. Sus-Mem showed, in addition to a much higher tensile strength ($p < 0.001$), a dense fibrillar structure.

Keywords: Guided bone regeneration, collagen membranes, Bio-Gide, Tisum Sus-Mem, degradation, tensile strength

Introduction

The rehabilitation of edentulism by means of osseointegrated implants has represented a revolutionary procedure within dentistry and has helped improve patients' quality of life and self-esteem [1]. In this regard, it has also been found that, following a tooth extraction, the alveolar ridge undergoes bone resorption and remodeling [2]. As a consequence, 50% of the initial volume of the extraction socket is resorbed within 12 months [3].

Guided bone regeneration (GBR) has become the therapeutic alternative for allowing implant placement in the appropriate position, where barrier membranes play an important role by preventing the invasion of soft tissue from the mucosa and creating an underlying space for bone growth [4]. Likewise, GBR facilitates the formation of a vascularized, functional bone structure that can support the load of dental implants [5].

In this study, two collagen membranes were considered: Geistlich Bio-Gide® and Tisum Sus-Mem. The Geistlich Bio-Gide® collagen membrane is a bilayer membrane derived from type I and III porcine collagen, free of artificial cross-linking [6]. Schröger *et al.* [7] described two sides to this membrane: the smooth side inhibits the growth of soft-tissue cells into the defect, while its rough side allows the growth of blood vessels and bone cells. Bio-Gide is recommended for alveolar ridge preservation after tooth extraction, for bone augmentation, and for maxillary sinus elevation [7].

In contrast, the Tisum Sus-Mem collagen membrane is a next-generation membrane that, similarly, has the capacity to stimulate bone regeneration and soft-tissue healing [8]. In addition, the Tisum Sus-Mem membrane undergoes the TISSUM TECH process, which allows purification of the collagen matrix of native pericardial tissue, yielding a

membrane free of antigenic compounds while preserving the structural integrity of the collagen fiber network [9].

The objective of this study was to compare *in vitro* the tensile strength and degradation of the Geistlich Bio-Gide and Tisum Sus-Mem collagen membranes [10].

The study hypothesis was that differences exist in the mechanical properties, hydrophilicity, and degradation between the Geistlich Bio-Gide and Tisum Sus-Mem membranes [11].

Materials and Methods

Sample Description

The study consisted of 68 samples of porcine-derived collagen membranes, including the Geistlich Bio-Gide® membrane from Geistlich Pharma AG, Switzerland, obtained from porcine dermis, and Sus-Mem® from Tisum-InBiomed, made from porcine pericardium. Their clinical use, biological origin, and the existing scientific evidence on their performance in GBR were reviewed in order to select them. The samples were distributed according to the experimental assay to be carried out: surface morphological analysis ($n=4$), hydrophilic property ($n=20$), mechanical properties ($n=8$) distributed into 2 groups, wet (saliva) and dry, and degradation test ($n=36$).

The independent variable of the study was the type of collagen membrane studied, either Geistlich Bio-Gide® or Tisum Sus-Mem®. The dependent variables, meanwhile, were surface morphology, elemental chemical composition, hydrophilic property, tensile strength, percentage deformation, and membrane degradation.

Sample Size Calculation

The sample size was calculated using the formula for finite populations, based on a population of 80 samples. A 95% confidence level, an expected proportion of 0.5, and a 5%

margin of error were applied. As a result, a sample of 66.35 was obtained, and for greater representativeness the sample was expanded to 68.

$$n = \frac{80(3.8416)(0.25)}{0.0025(79) + 0.9604}$$

$$n = \frac{76.832}{0.1975 + 0.9604}$$

$$n = \frac{76.832}{1.1579}$$

$$n = 66.35$$

Experimental Procedure

For the surface morphology analysis, the membranes were cut into 1 x 1 mm fragments and fixed onto a support with conductive adhesive, then coated with gold under vacuum. Evaluation of the prototypes was performed by scanning electron microscopy, using 100x and 1000x magnifications to observe the surfaces of both membranes.

For the hydrophilic property analysis, 5 x 5 mm prototypes were used, with five repetitions per membrane. This test was performed with an Optical Contact Angle Meter, depositing a drop of distilled water on the surface of each sample to record the contact angle and, in this way, obtain its hydrophilic behavior.

For the evaluation of the mechanical properties, the membranes were cut into 20 x 3 mm samples and evaluated using an Instron 34TM-5-SA Universal Testing Machine. In this evaluation, the membranes were subjected to continuous uniaxial tension until fracture, using a displacement rate of 5 mm per minute.

For the degradation analysis, 13 x 5 mm membranes were used, placed in 15 mL centrifuge tubes, where one group had artificial saliva and the other group had type I collagen solution. The samples were incubated for 7, 14, and 21 days at 37 °C, then freeze-dried and weighed on an analytical balance to determine the weight variation between the initial and final state.

Data Collection and Statistical Analysis

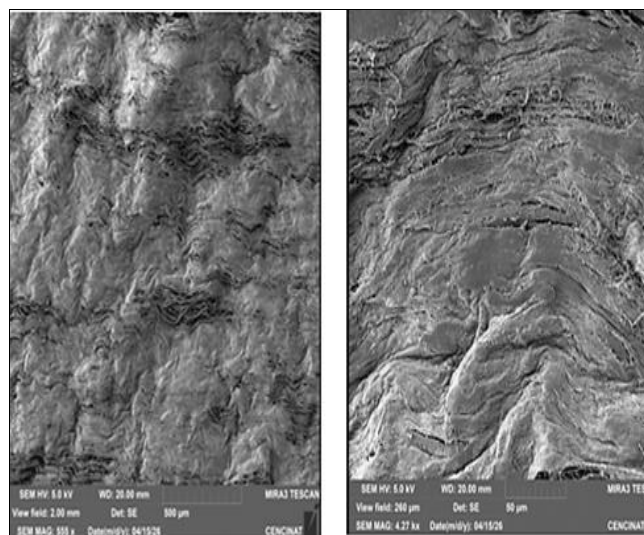
The data obtained in the surface morphology and chemical composition tests were analyzed descriptively based on the micrographs and elemental maps obtained by scanning electron microscopy and energy-dispersive spectroscopy (EDS). For the quantitative variables (contact angle, tensile strength, percentage deformation, and percentage weight loss in degradation), descriptive statistics (mean, standard deviation, and range) were calculated for each membrane. Before comparing the groups, the Shapiro-Wilk test, appropriate for small samples, was used to check the assumption of normality, and Levene's test was used to analyze the homogeneity of variances. Since the analysis compared only two independent groups (Bio-Gide and Tissum Sus-Mem), the independent-samples Student's t-test was used when the assumptions of homogeneity of variances and normality were confirmed. For the degradation analysis, given that each combination of incubation medium (artificial saliva or type I collagen solution) and evaluation time (7, 14, and 21 days) provided one value per membrane, allowing natural pairing between both groups, the paired-samples Student's t-test was used, following verification of the normality of the differences by means of the Shapiro-Wilk test. In all cases, p values below

0.05 were considered statistically significant, and the mean difference was reported together with its 95% confidence interval and the effect size using Cohen's d. Statistical analyses were performed using SPSS.

Results

All the collagen membrane samples were used and distributed according to the methodological requirements of each test performed.

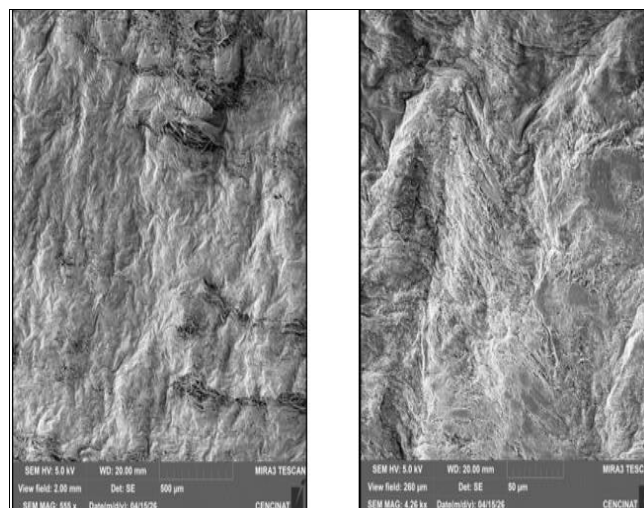
Surface Morphology Tests



Note: Authors' own work

Fig 1: Micrographs of the Bio-Gide membrane surface at different magnifications

Figure 1 shows micrographs of the Bio-Gide membrane obtained by scanning electron microscopy at different magnifications. A compact and dense collagen layer with fine fibers was observed.

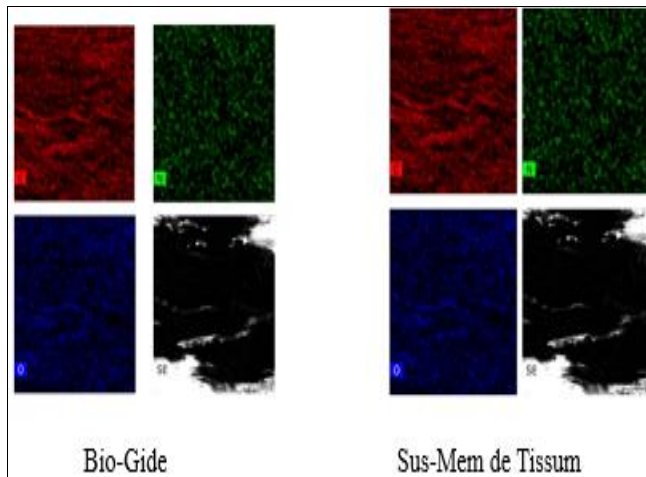


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Fig 1: Micrographs of the Tissum Sus-Mem membrane surface at different magnifications

Figure 2 shows micrographs of the Tissum membrane, obtained by scanning electron microscopy at different magnifications. It shows a denser, more compact structure. It presents collagen bundles oriented in multiple directions.

Chemical Composition Analysis (EDS)



Note: Authors' own work

Fig 2: Distribution of carbon (C), nitrogen (N), and oxygen (O), together with a secondary-electron (SE) image

Figure 3 presents elemental maps of carbon, nitrogen, and oxygen, together with a secondary-electron-mode micrograph of the Bio-Gide membrane; carbon was found to have a continuous distribution across the surface. Nitrogen and oxygen, on the other hand, were homogeneously distributed across the areas studied. In the SE-mode image, the surface structure of the membrane was observed, revealing a wavy arrangement of the fibers. Regarding Tissue Mem de Tissue, a similar distribution was observed, with a wavy fibrillar organization.

Hydrophilicity Tests

Table 1: Contact angle of the Bio-Gide and Tissue Mem de Tissue membranes on smooth and rough surfaces

Membrane	Surface	Measurements (°)	Mean (°)
Bio-Gide	Smooth	35.9; 37.1; 32.5; 30.7; 35.9	34.42
Bio-Gide	Rough	32.2; 34.0; 32.5; 30.7; 35.9	33.06
Tissue Mem de Tissue	Smooth	77.6; 71.9; 76.5; 78.4; 75.2	75.92
Tissue Mem de Tissue	Rough	75.9; 71.6; 74.2; 83.9; 70.6	75.24

Note: Authors' own work

Table 1 shows that, when evaluating the hydrophilic property, Bio-Gide obtained mean contact angles of 34.42° on its smooth side and 33.06° on its rough side. Tissue Mem de Tissue, on the other hand, showed higher values of 75.92° on its smooth side and 75.24° on its rough side, with greater dispersion in its measurements.

To determine whether the observed differences in contact angle were statistically significant, the Shapiro-Wilk test was applied to the values of each membrane on both surfaces, with no evidence of departures from normality ($p > 0.05$ in all cases), and Levene's test, which showed no significant differences in the homogeneity of variances (smooth surface: $F = 0.02$, $p = 0.891$; rough surface: $F = 1.45$, $p = 0.263$).

Consequently, an independent-samples Student's t-test was applied, which revealed statistically significant differences between the membranes on both the smooth surface ($t(8) = -25.01$; $p < 0.001$; mean difference = 41.50°; 95% CI: 37.67°–45.33°; Cohen's $d = 15.82$) and

the rough surface ($t(8) = -16.75$; $p < 0.001$; mean difference = 42.18°; 95% CI: 36.37° to 47.99°; Cohen's $d = 10.59$). These results statistically confirm that Bio-Gide exhibited greater hydrophilicity than Tissue Mem de Tissue on both surfaces.

Table 2: Statistical analysis of contact angle

Test	Statistic	P value	Interpretation
Shapiro-Wilk, Bio-Gide, smooth surface (n = 5)	W = 0.885	0.334	Normality assumption not rejected
Shapiro-Wilk, Tissue Mem de Tissue, smooth surface (n = 5)	W = 0.924	0.554	Normality assumption not rejected
Levene, smooth surface	F = 0.020	0.891	Homogeneous variances
Student's t-test, smooth surface (df = 8)	t = -25.01	< 0.001	Significant difference
Shapiro-Wilk, Bio-Gide, rough surface (n = 5)	W = 0.972	0.888	Normality assumption not rejected
Shapiro-Wilk, Tissue Mem de Tissue, rough surface (n = 5)	W = 0.877	0.294	Normality assumption not rejected
Levene, rough surface	F = 1.448	0.263	Homogeneous variances
Student's t-test, rough surface (df = 8)	t = -16.75	< 0.001	Significant difference

Note: Authors' own work

Membrane Tensile Tests

Table 3: Tensile strength

Membrane	Condition	Area (mm ²)	F (N)	TS (MPa)
Bio-Gide	Dry	1.05	1.84	1.75
Bio-Gide	Dry	1.05	2.33	2.22
Bio-Gide	Dry	1.05	2.53	2.41
Bio-Gide	Artificial saliva	1.05	1.06	1.01
Bio-Gide	Artificial saliva	1.05	1.27	1.21
Tissue Mem de Tissue	Dry	1.80	7.27	4.04
Tissue Mem de Tissue	Dry	1.80	6.90	3.83
Tissue Mem de Tissue	Dry	1.80	7.28	4.04
Tissue Mem de Tissue	Artificial saliva	1.80	9.35	5.19
Tissue Mem de Tissue	Artificial saliva	1.80	7.60	4.22

Note: Authors' own work

In Table 3, differences were observed in tensile strength between the Bio-Gide and Tissue Mem de Tissue membranes. Under dry conditions, Tissue Mem de Tissue obtained values of 3.83 MPa and 4.04 MPa, while Bio-Gide showed lower values of 1.75 MPa, 2.22 MPa, and 2.41 MPa. In artificial saliva, on the other hand, the Tissue Mem de Tissue membrane showed values of 5.19 MPa and 4.22 MPa, while Bio-Gide obtained 1.01 MPa and 1.21 MPa.

To determine whether the observed difference in tensile strength between the two membranes was statistically significant, the assumption of normality was assessed using the Shapiro-Wilk test, which showed no significant deviations in either group (Bio-Gide: $W = 0.925$, $p = 0.561$; Tissue Mem de Tissue: $W = 0.782$, $p = 0.057$). Levene's test likewise showed no significant differences in the homogeneity of variances ($F = 0.48$; $p = 0.510$), so an independent-samples Student's t-test with equal variances was applied. Statistically significant differences were found between the membranes ($t(8) = -7.01$; $p < 0.001$), with a mean difference of 2.55 MPa (95% CI: 1.71–3.39 MPa)

and a very large effect size (Cohen's $d = 4.43$), confirming that Tissum Sus-Mem exhibited significantly greater tensile

strength than Bio-Gide.

Table 4: Statistical analysis of tensile strength

Test	Statistic	p value	Interpretation
Shapiro–Wilk, Bio-Gide (n = 5)	W = 0.925	0.561	Normality assumption not rejected
Shapiro–Wilk, Tissum Sus-Mem (n = 5)	W = 0.782	0.057	Normality assumption not rejected
Levene	F = 0.476	0.510	Homogeneous variances
Student's t-test (df = 8)	t = -7.01	< 0.001	Significant difference

Note: Authors' own work

Table 5: Deformation

Membrane	Condition	Li (mm)	Lf (mm)	% Deformation
Bio-Gide	Dry	15	6.49	43.3
Bio-Gide	Dry	15	6.99	46.6
Bio-Gide	Dry	15	7.06	47.1
Bio-Gide	Artificial saliva	15	9.53	63.5
Bio-Gide	Artificial saliva	15	10.72	71.5
Tissum Sus-Mem	Dry	15	1.73	11.5
Tissum Sus-Mem	Dry	15	2.59	17.3
Tissum Sus-Mem	Dry	15	6.08	40.5
Tissum Sus-Mem	Artificial saliva	15	9.29	61.9
Tissum Sus-Mem	Artificial saliva	15	9.91	66.1

Note: Authors' own work

Analysis of the data obtained in Table 5 showed that, under dry conditions, the Tissum membrane presented values of 11.5%, 17.3%, and 40.5%, while the Bio-Gide membrane showed higher values of 43.3%, 46.6%, and 47.1%. When the membranes were exposed to artificial saliva, the Tissum membrane showed deformation values of 61.9% and 66.1%, while the Bio-Gide membrane had values of 63.5% and 71.5%. The initial length was kept constant at 15 mm for both membranes.

Similarly, the normality of the deformation values of each membrane was assessed using the Shapiro-Wilk test, with

no significant deviations observed (Bio-Gide: $W = 0.850$, $p = 0.194$; Tissum Sus-Mem: $W = 0.889$, $p = 0.354$), and variance homogeneity was assessed using Levene's test ($F = 2.34$; $p = 0.164$). When the independent-samples Student's t-test was applied, no statistically significant differences appeared in the percentage of deformation between the Bio-Gide and Tissum Sus-Mem membranes ($t(8) = 1.20$; $p = 0.265$), with a mean difference of 14.92 percentage points (95% CI: -13.79 to 43.63) and a moderate-to-large effect size (Cohen's $d = 0.76$).

Table 6: Statistical analysis of deformation

Test	Statistic	p value	Interpretation
Shapiro–Wilk, Bio-Gide (n = 5)	W = 0.850	0.194	Normality assumption not rejected
Shapiro–Wilk, Tissum Sus-Mem (n = 5)	W = 0.889	0.354	Normality assumption not rejected
Levene	F = 2.344	0.164	Homogeneous variances
Student's t-test (df = 8)	t = 1.20	0.265	No significant difference

Note: Authors' own work

Degradation Tests

Table 7: Initial and final weight of the Bio-Gide and Tissum Sus-Mem membranes at 7, 14, and 21 days

Day	Membrane	Medium	Initial weight (mg)	Final weight (mg)
7	Bio-Gide	Artificial saliva	22.7	20.9
7	Bio-Gide	Collagen solution	19.6	19.2
7	Tissum Sus-Mem	Artificial saliva	65.6	62.7
7	Tissum Sus-Mem	Collagen solution	51.1	47.5
14	Bio-Gide	Artificial saliva	19.5	14.1
14	Bio-Gide	Collagen solution	21.9	17.8
14	Tissum Sus-Mem	Artificial saliva	60.5	52.0
14	Tissum Sus-Mem	Collagen solution	53.6	44.9
21	Bio-Gide	Artificial saliva	31.2	27.9
21	Bio-Gide	Collagen solution	21.6	17.3
21	Tissum Sus-Mem	Artificial saliva	48.8	47.5
21	Tissum Sus-Mem	Collagen solution	63.2	58.7

Note: Authors' own work

Analysis of Table 7 showed that, at 7 days, Bio-Gide had final weights of 20.9 mg in artificial saliva and 19.2 mg in collagen solution, while Tissum Sus-Mem showed 62.7 mg and 47.5 mg. At 14 days, Bio-Gide recorded final weights of 14.1 mg and 17.8 mg, while Tissum Sus-Mem showed 52.0 mg and 44.9 mg. Subsequently, at 21 days, Bio-Gide had final weights of 27.9 mg and 17.3 mg, while Tissum Sus-Mem recorded 47.5 mg and 58.7 mg in artificial saliva and collagen solution.

Because each combination of incubation medium and evaluation time provided a single value per membrane, the observations were organized into corresponding pairs between Bio-Gide and Tissum Sus-Mem for each day-and-medium combination, and a paired-samples Student's t-test was applied. The Shapiro-Wilk test on the paired differences showed no departures from normality ($W = 0.94$; $p = 0.669$). The result of the paired t-test showed no statistically significant differences in the percentage weight loss between the membranes ($t(5) = -2.05$; $p = 0.096$; mean difference = -5.89 percentage points; 95% CI: -13.28 to 1.50), which was corroborated by the nonparametric Wilcoxon test ($p = 0.156$). In this analysis, although Bio-Gide showed a higher mean percentage weight loss (14.48%) than Tissum Sus-Mem (8.59%), this difference did not reach statistical significance at the conventional 0.05 threshold; however, the p value approached this limit.

Table 8: Statistical analysis of degradation

Test	Statistic	P value	Interpretation
Shapiro–Wilk of paired differences (n = 6)	W = 0.941	0.669	Normality assumption not rejected
Paired Student's t-test (df = 5)	t = -2.05	0.096	No significant difference ($\alpha = 0.05$)
Wilcoxon (nonparametric verification)	—	0.156	Corroborates no significant difference

Note: Authors' own work

Discussion and Conclusions

The results of this study were consistent with the findings of Shi *et al.*, who described significant differences in mechanical strength and elongation among collagen membranes of different origins [12]. In their study, membranes with higher tensile strength had lower elongation, while more flexible membranes had a greater capacity for deformation [13]. Peng *et al.* [14], on the other hand, found that the MC/Col membrane had a rougher, more granular surface compared with a conventional collagen membrane, and also found carbon, nitrogen, and oxygen homogeneously distributed [15]. In this study, both Bio-Gide and Sus-Mem showed a homogeneous distribution of carbon, nitrogen, and oxygen, with no alterations [16]. Regarding hydrophilicity, Shi *et al.* found differences in contact angles between the smooth and rough surfaces of the membranes they studied, describing lower angles in membranes with greater affinity for water [12]. In this study, Bio-Gide had lower contact angles than Sus-Mem, reflecting greater hydrophilicity and a better interaction with biological fluids and osteogenic cells [17]. Finally, Vallecillo *et al.* [18] also evaluated the degradative behavior of several collagen membranes, subjecting them to PBS and C. histolyticum collagenase, where they analyzed thickness and weight loss under different storage conditions. In that study,

they found differences among the membranes, with some showing complete degradation before 7 days and others by 21 days [19]. Among the limitations of the study was its *in vitro* nature, which did not allow the biological conditions present in the oral cavity to be fully reproduced [20]. Likewise, the study was carried out under controlled laboratory conditions, with observation over specific periods.

Regarding the conclusions, the Bio-Gide membrane showed a bilayer morphology with a marked difference between its smooth, compact surface and a rough, porous side. The Tissum Sus-Mem membrane, on the other hand, presented a denser structure with a fibrillar arrangement [21]. Understanding these structural differences and how they can influence the behavior of each membrane during guided bone regeneration processes was essential to the study [22].

When jointly analyzing the properties assessed, it is concluded that both membranes presented favorable characteristics for application in GBR; however, it should be noted that the Bio-Gide membrane stood out for its greater hydrophilicity ($p < 0.001$), while Tissum Sus-Mem was distinguished by its greater tensile strength ($p < 0.001$) [23]. Regarding the percentage of deformation and degradative behavior, the differences observed between the two membranes did not reach statistical significance [24].

However, when statistically comparing the percentage weight loss between Bio-Gide and Tissum Sus-Mem using a paired t-test, the difference between the two membranes did not reach statistical significance at the conventional 0.05 level ($t(5) = -2.05$; $p = 0.096$), although the p value approached that threshold [25].

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