

## Statistical Analysis

The variable of %OD was represented with mean and standard deviations. One-way analysis of variance (one-way ANOVA) was used to compare %OD between the different materials (K, S, E, L, I, C) within each group (No treatment, Thermocycling and Acidic Storage) at each day (day 1, day 3 and day 5) separately. Two-way repeated measures ANOVA was used to compare %OD between time (day 1, day 3 and day 5) and group within each material. Bonferroni corrections were made to adjust for multiple comparisons. Statistical analysis was performed using «IBM SPSS Statistics 28». Statistical significance level was set at  $p\text{-value} \leq 0.05$ .

## Results

**Table S1:** Mean %OD with standard deviation for each combination of group (No treatment, Thermocycling and Acidic Storage) and material (K, S, E, L, I, C) at each day.

Group	Material	Day 1	Day 3	Day 5	p-value
No treatment	K	108.17 (2.28)	136.42 (4.84)	105.73 (12.67)	0.072
	S	99.28 (5.64)	153.05 (13.15)	167.07 (7.73)	<0.001*
	E	104.88 (14.53)	71.54 (15.00)	118.28 (10.51)	<0.001*
	L	159.49 (1.92)	140.27 (1.95)	111.93 (12.10)	<0.001*
	I	103.12 (7.81)	153.56 (6.96)	131.12 (0.44)	<0.001*
	C	100.00 (3.96)	100.00 (1.27)	100.00 (3.79)	1.000
p-value		<0.001*	<0.001*	<0.001*	
Thermocycling	K	62.69 (5.89)	103.79 (12.22)	71.31 (0.14)	0.021*
	S	69.74 (6.84)	79.75 (0.68)	101.34 (5.16)	0.001*
	E	78.22 (13.45)	101.36 (2.63)	83.96 (5.23)	0.268
	L	98.24 (2.40)	118.78 (5.18)	113.27 (0.57)	<0.001*
	I	81.82 (0.84)	136.71 (6.11)	65.44 (9.67)	<0.001*
	C	100.00 (3.96)	100.00 (1.27)	100.00 (3.79)	1.000
p-value		<0.001*	<0.001*	<0.001*	
Acidic Storage	K	70.54 (3.24)	94.06 (21.97)	74.84 (3.94)	0.143
	S	71.42 (5.16)	102.32 (1.36)	97.90 (0.79)	0.002*
	E	75.82 (8.17)	109.33 (1.95)	63.10 (8.38)	<0.001*
	L	112.89 (15.61)	105.37 (11.03)	96.28 (11.67)	0.016
	I	77.34 (0.24)	134.62 (1.95)	81.34 (12.60)	<0.001*
	C	100.00 (3.96)	100.00 (1.27)	100.00 (3.79)	1.000
p-value		<0.001*	0.006	<0.001*	
*Statistically significant at level 0.05					

**Comparisons of %OD between the different materials (K, S, E, L, I, C) within each group (No treatment, Thermocycling and Acidic Storage) at each day separately.**

**1) No treatment**

**Day 1:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 1. More specifically, material L had greater %OD than the materials K ( $p$ -value $<0.001$ ), S ( $p$ -value $<0.001$ ), E ( $p$ -value $<0.001$ ), I ( $p$ -value $<0.001$ ) and C ( $p$ -value $<0.001$ ). No other significant differences were observed between the materials.

**Day 3:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 3. More specifically, material E had lower %OD than the materials K ( $p$ -value $<0.001$ ), S ( $p$ -value $<0.001$ ), L ( $p$ -value $<0.001$ ), I ( $p$ -value $<0.001$ ) and C ( $p$ -value $=0.031$ ). Material C had lower %OD than the materials K ( $p$ -value $=0.005$ ), S ( $p$ -value $<0.001$ ), L ( $p$ -value $=0.002$ ) and I ( $p$ -value $<0.001$ ). No other significant differences were observed between the materials.

**Day 5:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 5. More specifically, material S had greater %OD than the materials K ( $p$ -value $<0.001$ ), E ( $p$ -value $<0.001$ ), L ( $p$ -value $<0.001$ ), I ( $p$ -value $=0.006$ ) and C ( $p$ -value $<0.001$ ). No other significant differences were observed between the materials.

**2) Thermocycling**

**Day 1:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 1. More specifically, material L had greater %OD than the materials K ( $p$ -value $<0.001$ ) and S ( $p$ -value $=0.004$ ). Material C had greater %OD than the materials K ( $p$ -value $<0.001$ ), S ( $p$ -value $=0.002$ ) and E ( $p$ -value $=0.033$ ). No other significant differences were observed between the materials.

**Day 3:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 3. More specifically, material S had lower %OD than the materials K ( $p$ -value $=0.006$ ), E ( $p$ -value $=0.014$ ), L ( $p$ -value $<0.001$ ), I ( $p$ -value $<0.001$ ) and C ( $p$ -value $=0.023$ ). Material L had greater %OD than the material C ( $p$ -value $=0.040$ ). Material I had greater %OD than the materials K ( $p$ -value $<0.001$ ), S ( $p$ -value $<0.001$ ) and E ( $p$ -value $<0.001$ ). No other significant differences were observed between the materials.

**Day 5:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 5. More specifically, material K had lower %OD than the materials S ( $p$ -value $<0.001$ ), L ( $p$ -value $<0.001$ ) and C ( $p$ -value $<0.001$ ). Material S had greater %OD than the materials E ( $p$ -value $=0.022$ ) and L ( $p$ -value $<0.001$ ). Material E had lower %OD than the materials L ( $p$ -value $<0.001$ ) and C ( $p$ -value $=0.039$ ), and greater %OD than material I ( $p$ -value $=0.014$ ). Finally, material L had greater %OD than material I ( $p$ -value $<0.001$ ) and material I had lower %OD than material C ( $p$ -value $<0.001$ ).

**3) Acidic storage**

**Day 1:** there were significant differences in %OD between the 6 materials ( $p$ -value $<0.001$ ) at day 1. More specifically, material L had greater %OD than the materials K ( $p$ -value $<0.001$ ), S ( $p$ -value $<0.001$ ), E ( $p$ -value $=0.001$ ) and I ( $p$ -value $=0.002$ ). Material C had greater %OD than the materials K ( $p$ -value $=0.009$ ), S ( $p$ -

value=0.011) and E (p-value=0.038). No other significant differences were observed between the materials.

**Day 3:** there were significant differences in %OD between the 6 materials (p-value=0.006) at day 3. More specifically, material I had greater %OD than the materials K (p-value=0.005), S (p-value=0.031) and C (p-value=0.019). No other significant differences were observed between the materials.

**Day 5:** there were significant differences in %OD between the 6 materials (p-value<0.001) at day 5. More specifically, material E had lower %OD than the materials S (p-value=0.003), L (p-value=0.005) and I (p-value=0.002). No other significant differences were observed between the materials.

### **Comparisons of %OD between the different group (No treatment, Thermocycling and Acidic Storage) and days within each material (K, S, E, L, I, C).**

#### **1) Material K**

**Day 1:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 1. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value<0.001) and Acidic Storage (p-value<0.001). No significant difference was observed between Thermocycling and Acidic storage.

**Day 3:** there were significant differences in %OD between the 3 groups (p-value=0.029) at day 3. More specifically, No treatment group had greater %OD than group Acidic Storage (p-value=0.038). No other significant differences were observed between the groups.

**Day 5:** there were significant differences in %OD between the 3 groups (p-value=0.003) at day 5. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value=0.005) and Acidic Storage (p-value=0.008). No significant difference was observed between Thermocycling and Acidic storage.

**No treatment:** there were no significant differences in %OD between the 3 days (p-value=0.072).

**Thermocycling:** there were significant differences in %OD between the 3 days (p-value=0.021) at thermocycling group. More specifically, at day 3 the %OD was greater than day 1 (p=0.011) and day 5 (p=0.039). No significant difference was observed between day 1 and day 3.

**Acidic Storage:** there were no significant differences in %OD between the 3 days (p-value=0.143).

#### **2) Material S**

**Day 1:** there were significant differences in %OD between the 3 groups (p-value=0.001) at day 1. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value=0.003) and Acidic Storage (p-value=0.004). No significant difference was observed between Thermocycling and Acidic storage.

**Day 3:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 3. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value<0.001) and Acidic Storage (p-value<0.001). Moreover, Thermocycling group had lower %OD than Acidic Storage (p-value=0.033).

**Day 5:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 5. More specifically, No treatment group had greater %OD than

groups Thermocycling (p-value<0.001) and Acidic Storage (p-value<0.001). No significant difference was observed between Thermocycling and Acidic storage.

**No treatment:** there were significant differences in %OD between the 3 days (p-value<0.001) at No treatment group. More specifically, at day 5 the %OD was greater than day 1 (p<0.001) and day 3 (p=0.006). Moreover, day 3 had greater %OD than day 1 (p-value<0.001).

**Thermocycling:** there were significant differences in %OD between the 3 days (p-value=0.001) at thermocycling group. More specifically, at day 5 the %OD was greater than day 1 (p=0.001) and day 3 (p<0.001). No significant difference was observed between day 1 and day 3.

**Acidic Storage:** there were significant differences in %OD between the 3 days (p-value=0.002) at acidic storage group. More specifically, at day 1 the %OD was lower than day 3 (p<0.001) and day 5 (p=0.003). No significant difference was observed between day 3 and day 5.

### 3) Material E

**Day 1:** there were no significant differences in %OD between the 3 groups (p-value=0.051) at day 1.

**Day 3:** there were significant differences in %OD between the 3 groups (p-value=0.005) at day 3. More specifically, No treatment group had lower %OD than groups Thermocycling (p-value=0.019) and Acidic Storage (p-value=0.006). No other significant differences were observed between the groups.

**Day 5:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 5. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value=0.007) and Acidic Storage (p-value<0.001). No significant difference was observed between Thermocycling and Acidic storage.

**No treatment:** there were significant differences in %OD between the 3 days (p-value<0.001) at No treatment group. More specifically, at day 5 the %OD was greater than day 1 (p=0.015) and day 3 (p=0.008). No significant difference was observed between day 1 and day 3.

**Thermocycling:** there were no significant differences in %OD between the 3 days (p-value=0.268).

**Acidic Storage:** there were significant differences in %OD between the 3 days (p-value<0.001) at acidic storage group. More specifically, at day 5 the %OD was greater than day 1 (p=0.020) and day 3 (p=0.009). No significant difference was observed between day 1 and day 3.

### 4) Material L

**Day 1:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 1. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value<0.001) and Acidic Storage (p-value=0.002). No significant difference was observed between Thermocycling and Acidic storage.

**Day 3:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 3. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value=0.030) and Acidic Storage (p-value=0.003). No significant difference was observed between Thermocycling and Acidic storage.

**Day 5:** there were no significant differences in %OD between the 3 groups (p-value=0.136) at day 5.

**No treatment:** there were significant differences in %OD between the 3 days (p-value<0.001) at No treatment group. More specifically, at day 5 the %OD was lower than day 1 (p<0.001) and day 3 (p=0.004). Moreover, day 3 had lower %OD than day 1 (p-value<0.001).

**Thermocycling:** there were significant differences in %OD between the 3 days (p-value<0.001) at thermocycling group. More specifically, at day 3 the %OD was greater than day 1 (p<0.001). No other significant differences were observed.

**Acidic Storage:** there were significant differences in %OD between the 3 days (p-value=0.016) at acidic storage group. More specifically, at day 1 the %OD was greater than day 3 (p=0.017) and day 5 (p=0.044). No significant difference was observed between day 3 and day 5.

## 5) Material I

**Day 1:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 1. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value=0.004) and Acidic Storage (p-value=0.001). No significant difference was observed between Thermocycling and Acidic storage.

**Day 3:** there were significant differences in %OD between the 3 groups (p-value=0.010) at day 3. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value=0.028) and Acidic Storage (p-value=0.016). No significant difference was observed between Thermocycling and Acidic storage.

**Day 5:** there were significant differences in %OD between the 3 groups (p-value<0.001) at day 5. More specifically, No treatment group had greater %OD than groups Thermocycling (p-value<0.001) and Acidic Storage (p-value=0.002). No significant difference was observed between Thermocycling and Acidic storage.

**No treatment:** there were significant differences in %OD between the 3 days (p-value<0.001) at No treatment group. More specifically, at day 3 the %OD was greater than day 1 (p<0.001) and day 5 (p=0.019). Moreover, day 5 had greater %OD than day 1 (p-value<0.001).

**Thermocycling:** there were significant differences in %OD between the 3 days (p-value<0.001) at thermocycling group. More specifically, at day 3 the %OD was greater than day 1 (p<0.001) and day 5 (p<0.001). No significant difference was observed between day 1 and day 5.

**Acidic Storage:** there were significant differences in %OD between the 3 days (p-value<0.001) at acidic storage group. More specifically, at day 3 the %OD was greater than day 1 (p=0.017) (p<0.001) and day 5 (p<0.001). No significant difference was observed between day 1 and day 5.

## 6) Material C

**Day 1:** there were no significant differences in %OD between the 3 groups (p-value=1.000) at day 1.

**Day 3:** there were no significant differences in %OD between the 3 groups (p-value=1.000) at day 3.

**Day 5:** there were no significant differences in %OD between the 3 groups (p-value=1.000) at day 5.

**No treatment:** there were no significant differences in %OD between the 3 days (p-value=1.000).

**Thermocycling:** there were no significant differences in %OD between the 3 days (p-value=1.000).

**Acidic Storage:** there were no significant differences in %OD between the 3 days (p-value=1.000).