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A comparative evaluation of pH, ion release and apatite forming ability to assess bioactivity of a novel bioactive resin restorative dental material with a contemporary dental material – A multiparametric in-vitro study

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Abstract

Aim: To evaluate and compare bioactivity of ACTIVA BioActive Kids (AK) with GC Light Cured Universal Restorative (GC II LC) using the in vitro SBF bioactivity testing model.

Methods and Material: Six material disks of each material were soaked individually in 4 ml of SBF in sealed polyethylene tubes for preselected soaking time intervals (1, 7, 14 and 28 days). The material disks were evaluated for the apatite formation on the surface and the elemental analysis of precipitate via ESEM-EDX while the soaking solution was assessed for alkalizing ability and ion release profiles using a calibrated digital pH meter and ICP-OES, respectively. To describe the data, descriptive statistics, mean, and SD were used and were statistically analysed.

Results: AK demonstrated pH alkalizing property, significantly higher calcium and phosphate ion release and precipitate layer formation of varying thickness when compared to GC II LC for each predetermined time point. Significant differences were observed in the Ca-P ratio for the two test materials at all-time intervals.

Conclusions: AK appeared to possess bioactive properties in contrast to the bio-interactive GC II LC.

Keywords: ACTIVA™ BioActive Kids, Apatite forming ability, Bioactivity; Ca/P ratio; Ion release; pH; SEM-EDX spectrum; Simulated body fluid in vitro testing.

Introduction

Dental materials have been classified as bioinert (passive), bioactive, and bioresponsive or alternatively as 'smart materials', based on their interactions with the environment.[1] The ideal dental restorative material

should be bioactive, in addition to possessing good biocompatibility, optimal handling and setting properties. Bioactivity implies the ability to form an appetite layer which leads to superior bonding of restorative material to teeth and reduce microleakage by closure of interfacial gaps.[2] However, the known bioactive dental materials have low mechanical strength and are difficult to handle. Attempts have been made to develop materials that possess bioactivity and promote remineralization of the tooth by releasing calcium (Ca) and phosphate (P) ions.[3]

Resin modified glass-ionomer (RMGICs) were developed for improved resistance to microleakage and enhanced mechanical properties over conventional glass-ionomer cements (GIC), and retain the desirable qualities of the conventional version.[4,5] Resin based materials are durable and esthetic and widely used in restorative dentistry.[6] However, the polymerisation shrinkage on curing, generates contraction stresses that have a deleterious effect on the composite/tooth tissue interface.[7] Recurrent caries is a major mode of failure in composite restorations and efforts are constantly being made to formulate materials that will decrease its occurrence.[6] Areas of development in composite restorative materials include the incorporation of remineralizing agents into composites. The use of remineralizing materials in dentistry would prevent secondary caries due to a natural formation of apatite between material and tooth leading to a stable interface.[8]

ACTIVA™ BioActive Kids (AK), a novel bioactive resin-based material (enhanced RMGIC) reportedly stimulates apatite formation and the natural remineralization process with release and recharge of Ca, P and F. It is also reported to respond to pH cycles and play an active role in maintaining oral health with release and recharge of significant amounts of Ca, P and F.[9] These mineral components stimulate the mineral apatite crystal formation at the material-tooth interface, thus sealing the margins against microleakage and preventing secondary caries.[9,10]

However, there is paucity of research on the bioactivity of the enhanced RMGIC/ ionomeric resin, AK, restorative dental material in scientific literature. The objective of the present study was to evaluate and compare AK with a contemporary bio-interactive dental restorative material, GC Light Cured Universal Restorative (GC II LC). The null hypothesis was that there is no significant difference in the bioactivity of AK and GC II LC as assessed by comparing the ion release, apatite forming and pH modulating ability of the two materials.

Methods

Figure 1 outlines an overview of the experimental design employed for testing the pH, ion assessment and apatite forming ability of AK and GC II LC. Table 1 shows description, composition, and manufacturer details of the materials.

Table 1: Material description, composition and manufacturer details of the materials used in the study.

Material	Chemical Composition	Material description	Manufacturer	Batch number
ACTIVA™ BIOACTIVE KIDS [8].1 (5 ml) syringeACTIVA-SPENSER™ dispenser (5ml- 1:1) Automix tips with bendable 20 gauge 20-metal cannula	Blend of diurethane and other methacrylates with modified polyacrylic acid (44.6%) Amorphous silica (6.7%) Sodium fluoride (0.75%).	Novel bioactive resin restorative material (Enhanced RMGIC)	Pulpdent Corporation, Watertown, MA USA.	170109

GC Light Cured Universal Restorative [11,12]. Powder: 5g Liquid: 3g (2.6 ml) Powder scoop Mixing pad	Powder: Fluoro Aluminosilicate glass (amorphous) (90-100%). Liquid: Distilled water (20-30%), Polyacrylic acid (20-30%), 2-Hydroxyethylmethacrylate (30-35%), Urethanedimethacrylate <10, Camphorqunone <1.	Contemporary RMGIC	GC America Inc. Alsip, IL USA.	1708011

AK and GC II LC were mixed according to the manufacturer's instructions and transferred into the custom-made PVC mold (8mmx1mm±0.1)[13] and light cured for 20 seconds.[14] Twenty-four disks for each material were prepared. The average weight of the specimen disks was 0.04g (0.02-0.04g) and the surface area of each disk was approximately 125.6mm^2 [2(Πr^2)+2Πrh].[15] After polishing (1000 grit silicon carbide paper), the disks were washed with deionized water, and transferred for storage into sealed polyethylene tubes containing 4ml of SBF solution prepared using Kokubo and Takadama method.[13] Four sub-groups of six disks per material were constituted for analysis at preselected soaking time intervals. The investigator was blinded at this point to avoid bias**.** After the pre-determined endpoint times the samples were removed from the shaking bath, the material disks were extracted from the soaking solutions, rinsed gently with deionized water, and dried in an incubator at room temperature for 24h.

pH value of the soaking solution for each material disk was assessed using a calibrated Digital pH meter (Systronics India Ltd., Bengaluru). Four ml of the prepared SBF was used as a reference sample prior to starting the experiment (pH=7.4).[16] The electrode of the Digital pH meter was calibrated using 3M Potassium chloride maintained at the pH of 3.55-4.

After the pre-determined endpoint time, the soaking solution of each material disk was analyzed for ion release. The concentrations of Ca and P ions released in the solution were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (PerkinElmer Optima 5300 DE, Shiva Analyticals (India) Private Limited, Bengaluru).[16] Before the start of the experiment, two 4ml samples of the SBF solution were evaluated to establish the values of Ca and P.

After the pre-determined endpoint times, each material disk was subjected to ESEM-EDX (FEI make Quanta 200, Bengaluru) analysis. Each disk was placed on a conductive adhesive carbon strip mounted on an aluminum stub, sputter coated with gold using a Gold Sputter Coater (Baltec SCD 500 Sputter Coater, Bengaluru) and transferred to the ESEM to examine the apatite formation on the surface of the samples and precipitation of Ca-P under dry conditions. ESEM used accelerating voltage of 15–25 kV in a vacuum, with a working distance at the range of 9.4-10 mm and 0° tilt. SEM micrographs (secondary electron images) were obtained for all the specimens under 47x, 500x, 5000x, 10000x magnifications. The elemental analyses (weight% and atomic%) of samples were performed using EDX to characterize the surface composition of the disks by applying the standardless ZAF correction method. The surfaces areas of each disk were analyzed using 500x magnifications in mapping mode or by spot analysis.[16,17]

The collected data was analysed with IBM.SPSS statistics software 23.0 Version. To describe the data, descriptive statistics, mean and standard deviation (SD) were used. To find the significant difference in the

independent groups, the Mann-Whitney U test was used. For the multivariate analysis in repeated measures (Day 1,7,14 & 28) the Friedman test followed by the Wilcoxon signed rank test was used. In all the above statistical tools the probability value 0.05 was considered as significant level.

Results

Table 2-4 and Graph 1 shows comparative evaluation of the changes in the pH values of SBF soaking solution at different time intervals. GC II LC showed significantly lower pH values for each time interval ($p \leq 0.050$). The highest (7.25±0.03) and lowest pH (6.17±0.02) was on Day 1 and Day 28, respectively, with statistically highly significant (p=0.0005) overall difference. In AK, an overall statistically highly significant (p=0.002) increasing trend was observed with an insignificant decrease in the pH on Day 14 and the highest pH on the 28th day (7.78±0.09). The differences in the pH of the SBF soaking solution for GC II LC and AK were highly significant from Day 1 (p=0.009) to Day 7 (p=0.002) and on Day 28 (p=0.002), with AK consistently demonstrating significantly higher pH values than GC II LC. There was no significant difference between the groups on Day 14 (p=0.065).

Table 2: Intragroup comparison of pH of SBF for GC II LC and AK at predetermined soaking time points (in days) using Wilcoxon Signed Rank test.

Variable	Time interval in days	GC II LC (GROUP A)		AK (GROUP B)	
		Z^a	Asymp. Sig. (2-tailed)	Z^b	Asymp. Sig. (2-tailed)
pH	Day 1- Day 7	-2.201	.028	-2.201	.028
	Day 1- Day 14	-2.214	.027	-.943	.345
	Day 1- Day 28	-2.201	.028	-2.201	.028
	Day 7- Day 14	-2.214	.027	-.943	.345
	Day 7- Day 28	-2.201	.028	-2.207	.027
	Day 14- Day 28	-2.207	.027	-2.201	.028

a- Based on positive ranks.

b- Based on negative ranks.

Table 3: Overall intragroup comparison and mean values of pH of SBF for GC II LC and AK at predetermined soaking time points (in days) using Friedman's test

Group	Time interval in days	Mean ± SD	Mean Rank	Chi-Square	df	Asymp. Sig.
GC II LC (Group A)	1	7.25 ± 0.03	4.00	18.000	3	.0005
	7	6.99 ± 0.12	3.00			
	14	6.53 ± 0.40	2.00			
	28	6.17 ± 0.02	1.00			
AK (Group B)	1	7.31 ± 0.18	1.17	15.000	3	.002
	7	7.46 ± 0.18	2.17			
	14	7.22 ± 0.80	2.67			
	28	7.78 ± 0.09	4.00			

Table 4: Intergroup comparison of pH of SBF for GC II LC and AK at predetermined soaking time points (in days) using Mann- Whitney U test.

Variable	Time interval in days	Groups	N	Mean rank	Sum of ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)	Exact Sig. [2*(1-tailed Sig.)][b]
pH	1	GC II LC (Group A)	6	3.83	23.00	2	23	-2.57	0.01	0.009
		AK (Group B)	6	9.17	55.00					
		Total	12							
	7	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							
	14	GC II LC (Group A)	6	4.50	27.00	6	27	-1.92	0.054	0.065
		AK (Group B)	6	8.50	51.00					
		Total	12							
	28	GC II LC (Group A)	6	3.50	21.00	0	21	-2.89	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							

b- Not corrected for ties.

Table 5-9 and Graph 2 shows comparative evaluation of Ca and P ion release in the SBF solution. For GC II LC, the decrease in the Ca and P ion (p=0.028 for both Ca and P ion release) and overall difference (p=0.0005) in Ca and P ion release from Day 1 to Day 28 was statistically significant. For AK, the overall difference in the release of Ca and P ions on Day 1 and Day 28, was statistically highly significant (p=0.002). AK demonstrated a significantly higher Ca and P ion-release in comparison with GC II LC for each predetermined time point (p=0.002).

SEM images [Figure 3] with a 5000x magnification acquired prior to incubation in SBF, demonstrated differences in surface morphology when compared to after incubation. Material disks that were not soaked in SBF of both experimental groups GC II LC and AK demonstrated a homogenous and flat surface. EDX spectra for the same revealed elements of the cements. GC II LC without soaking in SBF showed the presence of Ca, Si, F, Al, Na, C, O, Nb peaks while AK showed the presence of Ca, P, Si, F, Al, Na, C, O, Au, Ba peaks.

Table 5: Intragroup comparison of calcium ion concentration in SBF for GC II LC and AK at predetermined soaking time points (in days) using Wilcoxon Signed Rank test.

Variable	Time interval in days	GC II LC (GROUP A)		AK (GROUP B)	
		Z[a]	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)
Calcium	Day 1- Day 7	-1.572	.116	-.135[a]	.893
	Day 1- Day 14	-1.992	.046	-2.201[a]	.028
	Day 1- Day 28	-2.201	.028	-2.207[b]	.027
	Day 7- Day 14	-1.572	.116	-1.782[a]	.075
	Day 7- Day 28	-2.201	.028	-2.201[b]	.028
	Day 14- Day 28	-1.363	.173	-2.207[b]	.027
Phosphate	Day 1- Day 7	-1.992[c]	.046	-1.892[b]	.058
	Day 1- Day 14	-2.201[c]	.028	-2.201[a]	.028
	Day 1- Day 28	-2.201[c]	.028	-.734[a]	.463
	Day 7- Day 14	-1.363[c]	.173	-2.201[a]	.028
	Day 7- Day 28	-2.201[c]	.028	-1.153[a]	.249
	Day 14- Day 28	-2.201[c]	.028	-2.201[b]	.028

a- Based on positive ranks.

b- Based on negative ranks.

Table 6: Intragroup comparison of phosphate ion concentration in SBF for GC II LC and AK at predetermined soaking time points (in days) using Wilcoxon Signed Rank test.

Variable	Time interval in days	GC II LC (GROUP A)		AK (GROUP B)	
		Z[a*]	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)
Phosphate	Day 1- Day 7	-1.992	.046	-1.892[b**]	.058
	Day 1- Day 14	-2.201	.028	-2.201[a*]	.028
	Day 1- Day 28	-2.201	.028	-.734[a*]	.463
	Day 7- Day 14	-1.363	.173	-2.201[a*]	.028
	Day 7- Day 28	-2.201	.028	-1.153[a*]	.249
	Day 14- Day 28	-2.201	.028	-2.201[b**]	.028

*a- Based on positive ranks.

**b. Based on negative ranks.

Table 7: Overall intragroup comparison of Ca and P ions concentration in SBF for GC II LC and AK at predetermined soaking time points (in days) using Friedman's test.

Group	Variable	Time interval in days	Mean ± SD	Mean Rank	Chi-Square	df	Asymp. Sig.
GC II LC (Group A)	Calcium	1	4.69 ± 0.95	3.67	11.800	3	0.008
		7	3.65 ± 0.76	2.83			
		14	3.01 ± 0.68	2.33			
		28	2.56 ± 0.35	1.17			
AK (Group B)		1	10.40 ± 0.37	2.58	15.000	3	0.002
		7	10.37 ± 0.31	2.25			
		14	10.02 ± 0.33	1.17			

		28	11.24 ± 0.50	4.00			
GC II LC (Group A)	Phosphate	1	3.21 ± 0.18	3.83	15.800	3	0.001
		7	2.24 ± 0.62	3.00			
		14	1.71 ± 0.51	2.17			
		28	0.90 ± 0.00	1.00			
AK (Group B)		1	4.61 ± 0.29	2.83	13.400	3	0.004
		7	5.23 ± 0.93	3.67			
		14	3.61 ± 0.31	1.00			
		28	4.85 ± 1.32	2.50			

Table 8: Intergroup comparison of calcium ion concentration in SBF for GC II LC and AK at predetermined soaking time points (in days) using Mann- Whitney U test.

Variable	Time interval in days	Groups	N	Mean rank	Sum of ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)	Exact Sig. [2*(1-tailed Sig.)][b*]
Calcium	1	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							
	7	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							
	14	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							
	28	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							

*b- Not corrected for ties.

Table 9: Intergroup comparison of phosphate ion concentration in SBF for GC II LC and AK at predetermined soaking time points (in days) using Mann- Whitney U test.

Variable	Time interval in days	Groups	N	Mean rank	Sum of ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)	Exact Sig. [2*(1-tailed Sig.)][b*]
Phosphate	1	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							

	7	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							
	14	GC II LC (Group A)	6	3.50	21.00	0	21	-2.88	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							
	28	GC II LC (Group A)	6	3.50	21.00	0	21	-3.08	0.004	0.002
		AK (Group B)	6	9.50	57.00					
		Total	12							

*b- Not corrected for ties.

On Day 1, surface of GC II LC disks showed sparsely distributed, very thin precipitate layer while AK demonstrated a sparsely distributed but visibly thicker precipitate layer [Figure 4]. On Day 7, GC II LC showed small agglomerate structures while AK disks demonstrated a thicker precipitate layer with larger agglomerate structures [Figure 5]. GC II LC demonstrated a thin layer of precipitate like the previous time intervals on Day 14 while AK showed a thicker precipitate layer with larger and dense agglomerate structures [Figure 6]. Day 28 showed precipitates on the surface confined only to a limited area on the GC II LC disks. In contrast, AK group demonstrated a combination of thick and thin precipitate layer covering half to more than half of the disks [Figure 4]. Additionally, cracks were observed for all disks of GC II LC on Day 28 which were absent on previous time intervals and for AK group. The EDX spectrum showed that the main elements in the precipitate on the disk after immersion in SBF for all time intervals, were Ca, P, Si, F, Al, C, O, Na & Au for both GC II LC & AK.

Table 10 and Graph 2 depicts comparative evaluation of Ca-P ratio. GC II LC showed Ca-P ratio at the range of 0.06-0.73 while AK demonstrated a Ca-P ratio in the range of 3.38-1.82. The two experimental groups demonstrated statistically significant difference for Day 1 (p=0.024) and statistically highly significant difference for Day 7, 14 and 28 (p=0.017, 0.004, 0.010 respectively) i.e., Ca-P ratio for AK was higher at all time points when compared with GC II LC.

Table 10: Mean, Standard deviation and intergroup comparison of calcium-phosphate ratio of GC II LC and AK using Mann Whitney U test.

Time interval in days	GC II LC (Group A)		AK (Group B)		Mann-Whitney U	Z	Asymp. Sig. (2-tailed)	Exact Sig. [2*(1-tailed Sig.)]
	Mean	SD	Mean	SD				
1	0.72	0.45	3.38	1.28	0.000	-2.32	0.020	0.024[b]
7	0.73	0.91	2.87	0.88	2.000	-2.37	0.018	0.017[b]
14	0.11	0.04	2.77	0.76	0.000	-2.73	0.006	0.004[b]
28	0.06	0.02	1.82	0.57	0.000	-2.55	0.011	0.010[b]

P - Value	** Highly Significant at P ≤ 0.01
P - Value	* Significant at 0.01 < P ≤ 0.050
P -Value	# No Significant at P > 0.050

Discussion

Bioactive materials, upon contact with body fluid are known to promote the formation of hydroxyapatite (HA) on the surface and between the tooth structure and the restorative material, closing the gaps between the materials and tooth, thereby enhancing the tooth integration with the restoration, while also promoting remineralization of the tooth by releasing Ca and P ions.[2,13,15,18,19-29]

ACTIVA™ BioActive is reportedly the first durable, esthetic, bioactive restorative material, suitable for both dentin and enamel replacement.[9,30,31] ACTIVA is considered a RMGI[32,33] as it contains two acids, it is also referred as ionic resin composite[28,29,34-36] since the chemical cure is not only a GI reaction but also combined with the self-cure resin composite. The description "ionic" was based on ionization process of the P group between the resin and glass filler at one side and the tooth structure on the other side, as the hydrogen ions break off from the P groups and are replaced by Ca in tooth structure, forming an ionic bond between the filling and the tooth structure.[9,31,34] ACTIVA also contains a bioactive filler making it bioactive and able to form a HA layer. Based on these facts, ACTIVA is best described as a "Resin-modified GI bioactive ionic resin-based composite", or simply "ionic bioactive resin material".[34]

The present study, designed to evaluate and compare the bioactivity demonstrated the superior bioactive properties of ACTIVA™ BioActive Kids when compared to the bio-interactive GC Light Cured Universal Restorative.

GC II LC Group showed a gradual, statistically significant decline in the pH values of SBF soaking solution, Ca and P ion release and developed negligible amount of precipitate which can be attributed to the release of the unreacted polyacrylic acid from the sample.[13,16,37,38] It can thus be derived that GC II LC released insufficient Ca and P ions that are necessary to form a HA precipitate layer and is therefore inherently bio-interactive rather than bioactive. A similar study investigating the solution buffering and ion-release of resin modified glass-ionomers in water (pH-5.3) and aqueous lactic acid (2.7) over 6 weeks showed P release to be more in lactic acid than in water.[27] A study examining the activity and ability of two glass ionomer cements and a compomer to mineralize teeth in gaps, observed hemispherical-shaped precipitations composed of minute semicircle plate-like crystals on the enamel surface after one year of water storage for glass ionomer cement. The amount of crystal growth in chemical-cured type of glass ionomer cement was greater than in dual-cured type of glass ionomer cement with no crystal formation in the compomer.[39] In the present study, AK Group displayed a gradual, statistically significant increase in pH values of SBF soaking solution and an overall Ca and P ion release that led to the formation of a thick layer of precipitate on the material disks. Study reports sustained Ca ion release in Dulbecco's phosphate-buffered saline (D-PBS) solution over 21 days from ACTIVA. ACTIVA showed greater variance by releasing the most ions into solution in comparison with other tested materials with an initial ion value of 25.1mg/L and maintaining 17.0mg/L at 21 days. Thus, the Ca release for ACTIVA might suggest an ability to promote tooth remineralization.[40] The cumulative amount of P release from ACTIVA in 7 days period was around 300 mcg/g in pH 4, and 100 mcg/g in pH 7, indicating that ACTIVA exhibits different behavior according to the acidity of the environment.[28] The uptake of P ions from solution as the amorphous calcium phosphate (ACP) layer crystallizes, results in the drop in

P ion concentration. The effect becomes pronounced when the ACP film turns to crystalline apatite.[41]

In the present study the precipitate on the AK disk surface did not deplete over time and showed a gradual increase, it can therefore be presumed the precipitate formed was a stable compound of Ca-P. Incidentally, GC II LC Group showed cracks on Day 28, a finding not visualized during the previous time intervals. The surface of the material disks of AK Group remained intact. This feature may point towards AK possessing better moisture tolerance in comparison with GC II LC, on account of its hydrophilic nature[9,31,35] and unique chemistry.[9,31]

In a profilometry analysis and evaluation, ACTIVA demonstrated Ca-P ratio in the range of 2.0-2.5 across dentin, tags and resin.[42] EDX results indicates that Ca precipitation in GC II LC was less than that of AK cement. The Ca-P depositions on the surface of AK appear to be either Ca-rich non-apatitic, Ca-P which are known to have the Ca-P ratio of 1.83 or Ca-rich (carbonated) apatite which demonstrate Ca-P ratio between 1.6-2.00.[43] A similar study with mineral apatite deposits on the surface of specimen disks immersed in DPBS for 14 and 28 days appeared structurally similar in morphology to the precipitate on the AK disks in the present study.[44]

High pH levels (7.5 or higher) appear to stimulate more active and complete bioactivity.[45] It is believed that increase of solution pH benefits the apatite nucleation since apatite solubility decreases at basic pH and OH is required to form apatite. This change of pH can facilitate the formation of apatite nucleation on the cement surface.[13] Studying this phenomenon provides a greater insight into the ion transfer which can take place between the material disk surface and the SBF.[46] The increase in the pH values of the SBF soaking solution demonstrated by AK can be attributed to the ion exchange process. Hench reported that the change of pH can facilitate the formation of apatite nucleation on the material surface and the release of Ca provided enough ions for the apatite crystal to grow. The formation of the Ca-P layer on the surface of samples is the main indicator of bioactivity. Changes in the Ca concentration correlated with changes in the P concentration confirm the precipitation of Ca-P.[46]

The slight drop in pH of the SBF solution for the AK group and the simultaneous drop in Ca and P ion release on Day 14, followed by an increased release on Day 28 can be attributed to apatite nucleation on the disk surface resulting from the super saturation of the solution with Ca and P ions leaching from the material disk. The decrease in the P concentrations is likely due to the precipitation of the Ca-P layer, which consumes Ca and P ions from the SBF medium.[46-48] The precipitation of HA from highly and medium supersaturated solutions at pH >7 is preceded by the formation of ACP, which transforms after a reproducible induction period into a crystalline apatitic phase. The conversion rate has been shown to increase with pH in the range 7-10, and then to decrease at higher pH.[49] In the present study, AK demonstrated pH values >7 at all-time intervals. Studies confirm the rapid increase in pH in the first stage (between 1 and 7 days) is due to the leaching of cations out of samples, which are further exchanged with H^+ ions from the solution. In later stage (between 7 and 28 days), the pH value increases very slowly in comparison with the first stage resulting in the formation of HA layer on the surface of the samples.[46] Hench observed that the ion exchange process leads to an increase in interfacial pH with time of immersion, to values >7.4.[46,48] ACTIVA reportedly maintained pH at a neutral and consistent level over the time.[40]

The limitation of the present study includes the use of SBF in-vitro test model for bioactivity as this test can generate both false negative and false positive results. The SBF in vitro assay for bioactivity cannot completely simulate oral conditions.[18,50] Therefore, in vivo tests should be conducted to validate the results as per recommendation.[18] The present study was conducted only under static and not dynamic regime. Additionally, the topographic analysis of nucleation rate, morphology, composition, crystallinity of Ca-P precipitate with FTIR, Raman spectroscopy, interferometer was beyond the scope of the present study and is recommended in future research.

Conclusion

The results of the study demonstrate significant differences between the two groups, with AK displaying higher pH, releasing significantly higher Ca and P ions, and producing precipitates with higher Ca-P ratios than GC II LC. Within the limitations of the present study, it can be inferred that, AK possesses bioactivity due to its inherent ion releasing property, apatite forming and concurrent pH alkalizing abilities. AK can therefore be considered a suitable alternative to contemporary dental restorative materials. However, further research is recommended to evaluate its performance in the clinical scenario.

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Legend Figures

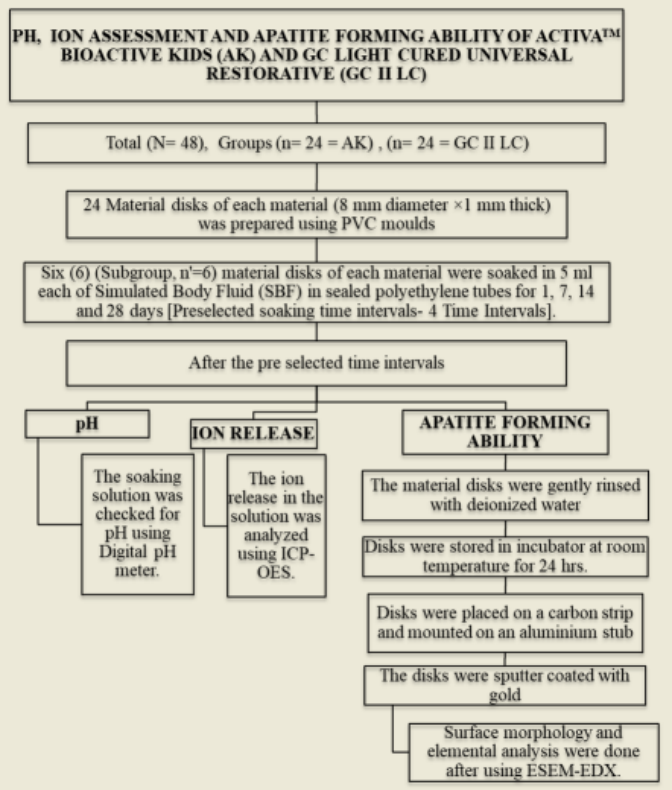


Figure 1: An overview of the experimental design

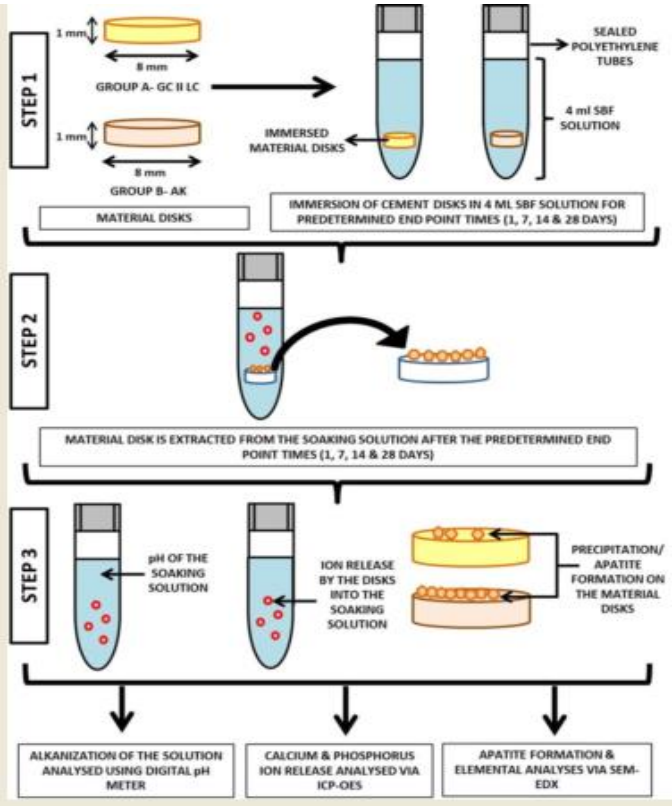


Figure 2: Experimental design of in vitro SBF bioactivity testing.

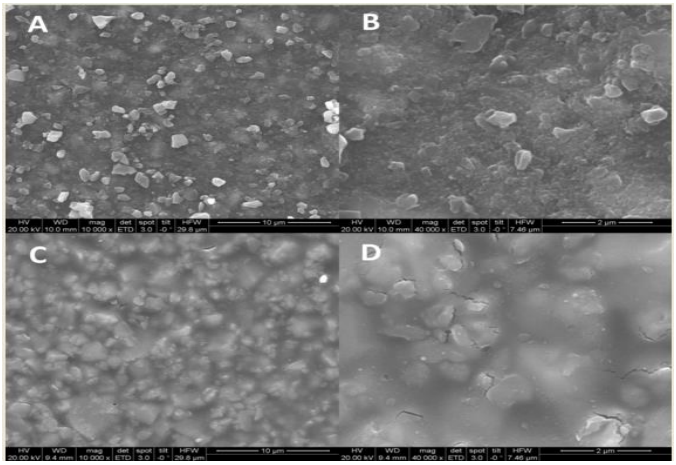


Figure 3: SEM micrograph of the surface morphology of AK (A, B) and GC II LC (B, C) without soaking in SBF.

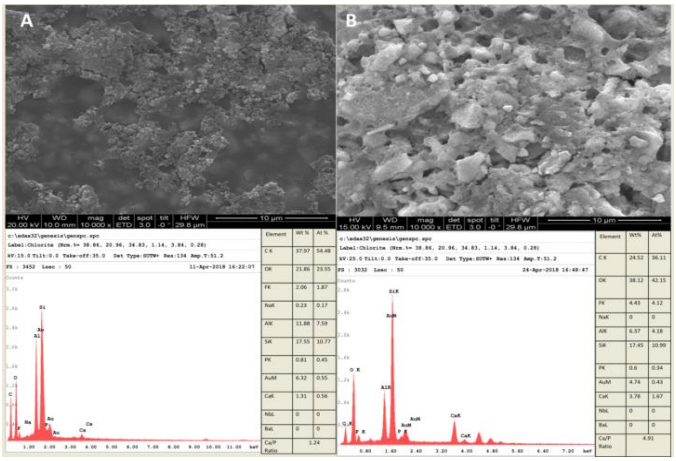


Figure 4: Representative SEM photomicrographs and EDX elemental analysis of GC II LC (A) and AK (B) on Day 1 after immersion in SBF.

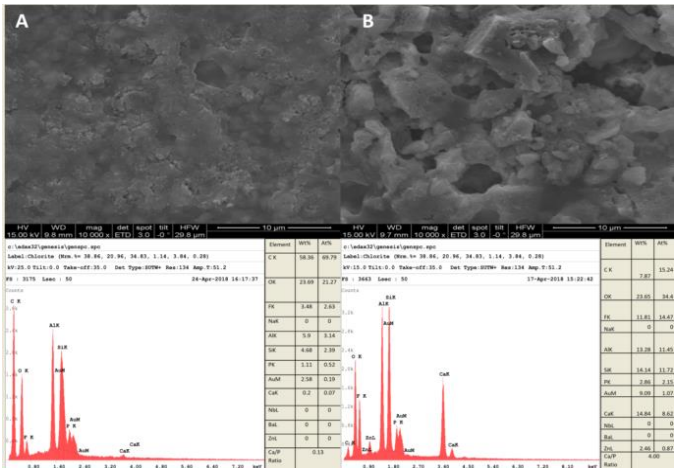


Figure 5: Representative SEM photomicrographs and EDX elemental analysis of GC II LC (A) and AK (B) on Day 7 after immersion in SBF.

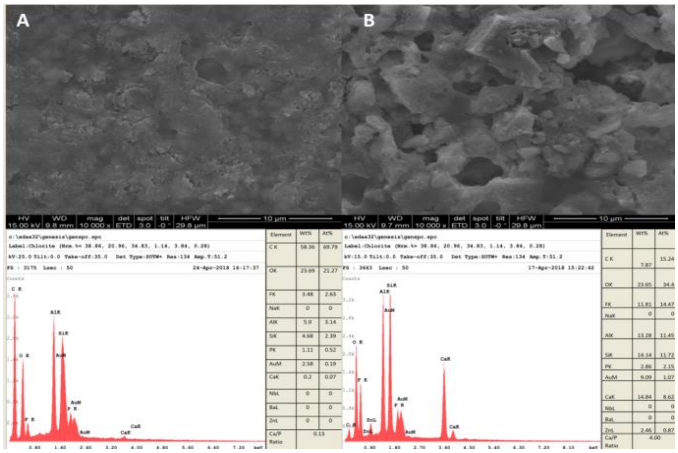


Figure 6: Representative SEM photomicrographs and EDX elemental analysis of GC II LC (A) and AK (B) on Day 14 after immersion in SBF.

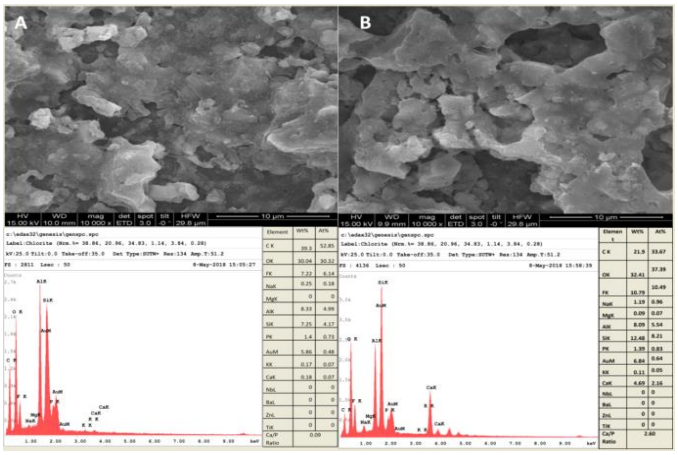
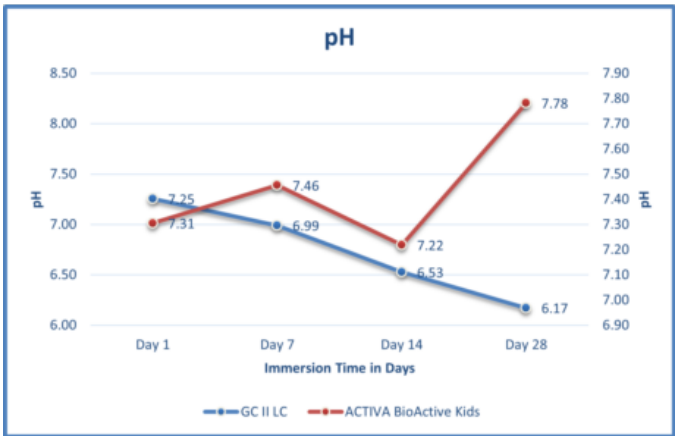
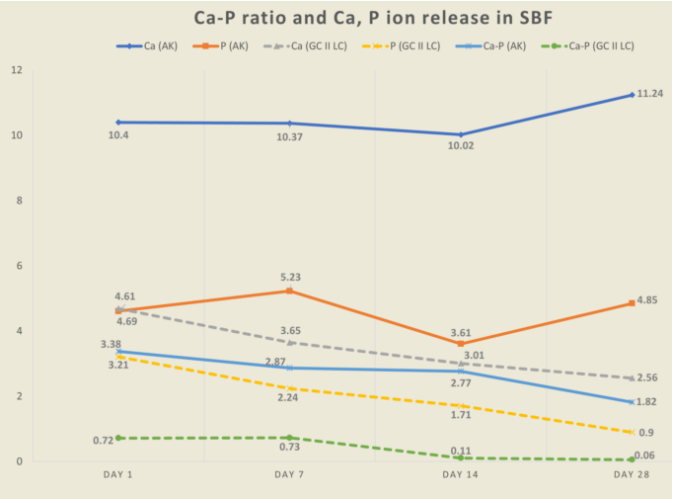


Figure 7: Representative SEM photomicrographs and EDX elemental analysis of GC II LC (A) and AK (B) on Day 28 after immersion in SBF.



Graph 1: Mean values of pH of the SBF solutions for GC II LC and AK after soaking for various periods of

time (1, 7, 14 and 28 days) without refreshing the soaking medium.



Graph 2: The changes of the concentration of Ca and P ions in the SBF soaking solution and Ca-P ratio for GC II LC and AK after soaking for various periods of time (1, 7, 14 and 28 days) without refreshing the soaking medium.