ORIGINAL ARTICLE

ANALYSIS OF REMINERALIZING CHEWING GUM CONTAINING BIOACTIVE GLASS – A SCANNING ELECTRON MICROSCOPIC STUDY

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ABSTRACT

The objective of this study was to identify components and distribution of bioactive (BAG) particles in remineralizing chewing gum by using scanning electron microscopy (SEM). Bioactive glass containing SiO2-P2O5-CaO-Na2O-CaF2 was prepared by melt quench route and the obtained powdered glass was mixed with a chewing gum base. The chewing gum either had 2.5% or 5% bioactive glass. Manual chewing was done for 10 mins by immersing the chewing gum in tris-buffer in a nitrile glove cut finger and applying steady pressure with fingers of one hand. The chewed gum specimens after freezing with liquid nitrogen were observed under SEM and its composition was determined with energy-dispersive x-ray spectroscopy (EDS). By using SEM/EDS the various elements such as calcium, oxygen, fluoride, phosphate, carbonate and silica were identified in gum base containing BAG. Moreover, the glass particles were randomly distributed in gum base before and after chewing. The glass particles dissolved in tris-buffer solution release ions after chewing which might have tendency to form fluorapatite (FAP) important for remineralization. These particles are randomly distributed in chewing gum base though further studies are warranted with refinement.

Key words: *bioglass, calcium, caries resistance, carbonate, fluoride, fluorapatite*

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INTRODUCTION

Enamel and dentin are the mineralized components of a tooth making the external surface and the bulk, protecting the underlying vascularized and neural tissue of the dental pulp. Enamel is composed of millions of hydroxyapatite crystals composed of calcium phosphate $Ca_{10}(PO_4)_6(OH)_2$ and trace elements such as carbonate, strontium, magnesium, fluoride and lead.[1] Enamel cannot regenerate itself after becoming demineralized due to acid released from fermentation of carbohydrates by bacteria in the dental plaque. Demineralization of enamel and/or dentin can lead to caries development. Enamel relies on the presence of calcium, phosphate, and fluoride ions in the saliva to remineralize itself. Extensive research is being done to look for ways to prevent caries process and to avoid extensive dental procedures. Fluoride plays an important role in this regard.[2] Fluoride ions have more affinity for hydryoxyapatite crystals, replacing the hydroxyl ions with fluoride ions to form fluorapatite (FAP)[1] which is more resistant to acidic demineralization and inhibits bacterial growth and metabolism.[3] Fluoride can be added in tooth paste, dental varnishes, mouth washes, and chewing gums to aid in enamel remineralization. Chewing gum can be an effective carrier source of remineralizing ions such as fluoride which can decrease enamel solubility and promote remineralization by ion precipitation on enamel surface.[4] It also increases salivary flow rate and pH of saliva which is important in cleansing of food debris and bacteria from the oral cavity. On chewing fluoride-rich gum, fluoride levels of saliva rise in the initial 5-10 min followed by a gradual decrease.[5]

BAG are called as calcium-phospho-silicates because on dissolving in physiological solutions they release calcium and phosphate ions which promote

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hydroxy-carbonated-apatites which are similar to tooth minerals.[6] BAG are biocompatible and are being used in bone grafts[7], dentifrices, and tooth pastes,[8] and have helped with treatment of dentine hypersensitivity.[8] NovaMin BAG has shown the potential of reminerazling dentin specimens and was able to occlude the dental tubules with hydroxyapatite-resembling crystal structure.[9]

SEM is a useful tool in material research which shows the morphology and characterization of different materials. The narrow electron beam is produced from a tungsten filament and is focused on a given specimen and the signals are collected. The evidence about composition of specimen can be obtained by back scattered secondary electrons and microprobe that gathers the emitted x-rays. As each element consists of characteristics wave length for x-rays so the type and quantity of each element in a specimen such as a chewing gum can be analyzed by x-ray spectra emission which is collected with EDS microprobe.[10, 11]

In this study, fluoride containing BAG was incorporated into a chewing gum base and gum base was used as a carrier vehicle. Chewing gums have been used in the past as carriers for fluoride. [5, 12] SEM was done to confirm the presence of different elements in BAG particles before and after chewing in gum base. Some ions calcium, uoride, phosphorus may dissolve from BAG into the solution. Thus, the concurrent release of fluoride with calcium and phosphate ions can enhance FAP formation necessary for remineralization. Hence, the purpose of this study was to see various components of BAG present in chewing gum base that might have released ions in physiological solutions during chewing process capable for enamel remineralization.

METHODOLOGY

BAG as developed by Mneimne *et al.*[13] was prepared by first calculating the mol% of the glass components as given in table 1, followed by mixing of calcium carbonate, calcium fluoride, sodium carbonate, silica, and phosphate to make a batch of 200 g.

After melting the BAG in electric furnace (EHF 17/3, Lenton, Hope Valley, UK) at 1430°C for 1 hour, the molten glass was quenched in cold water and then dried at 37°C in drying cabinet. Powder was made from this glass frit using vibratory mill (Gyro mill, Glen Creston, London, UK). This powder was sifted with a 38 µm sieve (Endecotts Ltd, London, UK) and collected. Four batches were prepared similarly. 250 g of this fine BAG was incorporated into chewing gum bases in different weightage hence obtaining 2.5% BAG low gum base (GB), 5% BAG low GB, 2.5% BAG high GB, and 5% BAG high GB for analysis that which combination yields better results.

Two liters of tris-buffer (pH 7.36) was prepared and stored at 37C in anlagen bottle. At the time of manual chewing experiment, 5 ml was taken in a falcon tube and the chewing gum specimen was place inside. A finger was cut from nitrile glove and tied on the rim of this falcon tube which is then fixed upside down on a stand. Manual chewing of the specimen was done with fingers of one hand by applying constant force one every two seconds apart for 10 mins. [14]

One specimen randomly selected was taken from each gum base before chewing and the same were analyzed with SEM/EDS after chewing. For SEM/EDS analysis, the specimens were frozen in liquid nitrogen for 1-2 mins and then cut. Specimens were coated with conductive carbon cement for one day before analysis.

RESULTS

SEM/EDS analysis before chewing

The gum bases containing BAG were observed under SEM/EDS to check for the dispersion of the glass particles. Along with the components of glass particles, this can also identify the presence of $CaCO_3$ and talc or mica (figure 1, tables 2-6).

SEM/EDS after chewing

DISCUSSION

The SEM analysis before chewing of 2.5% and 5% BAG low GB (figure 1) show the presence of calcium, oxygen, and carbonate. 2.5% BAG low GB spectrum 2 specify the presence of glass particles in GB (table 2). However, 5% BAG low GB spectrum 4 shows magnesium, silicon, and aluminum and most likely represent the presence of talc or mica in GB (table 3). SEM analysis 2.5% BAG high GB in spectrum 3 is showing carbon, oxygen, and calcium whereas spectrum 4 is showing silicon along with carbon, oxygen and calcium (figure 1, table 4). 5% BAG high GB spectrums 2 and 5 are showing the presence of carbon, oxygen, and calcium whereas spectrum 3 is detecting the presence of glass particles (figure 1, table 5). Hence, this demonstrates the random distribution of glass particles along with calcium carbonate in the GB. The SEM/EDS is done after

TABLE 1: COMPOSITION OF BIOACTIVE GLASS

Serial No	Bioactive glass components	Mol%
1	SiO2	36.41
2	Na2O	28.28
3	CaO	24.74
4	P2O5	6.04
5	CaF2	4.53
6	NC	2.08

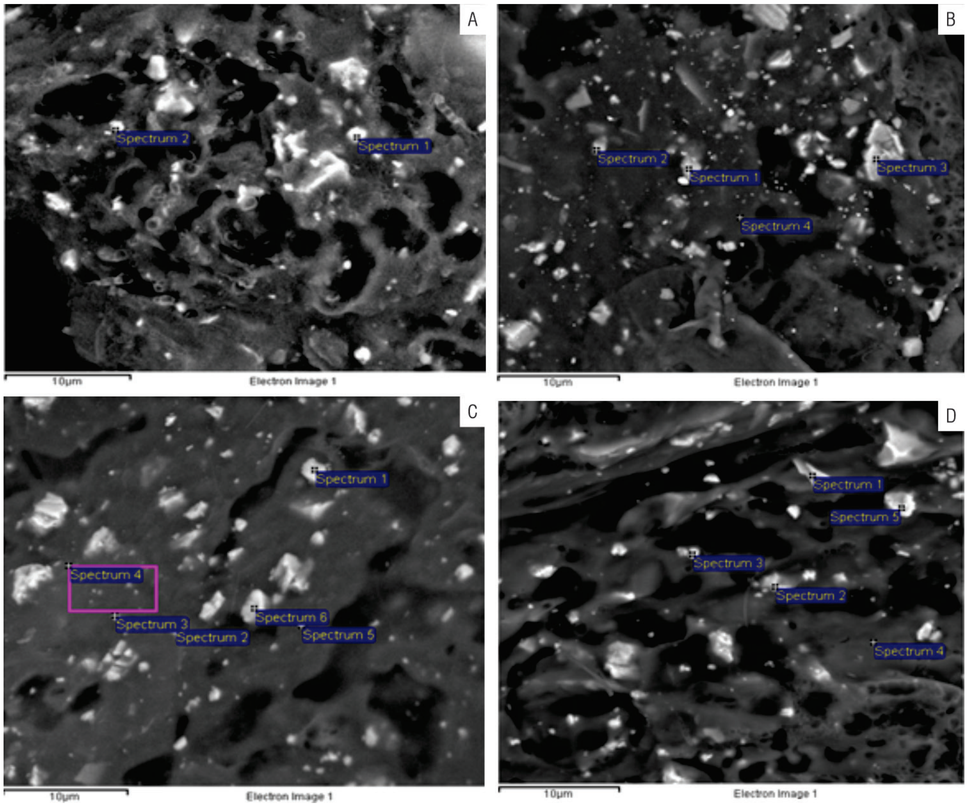

Fig 1: SEM of chewing gum before chewing experiment. (A) 2.5% BAG low GB, (B) 5% BAG low GB, (C) 2.5% BAG high GB, (D) 5% BAG high GB

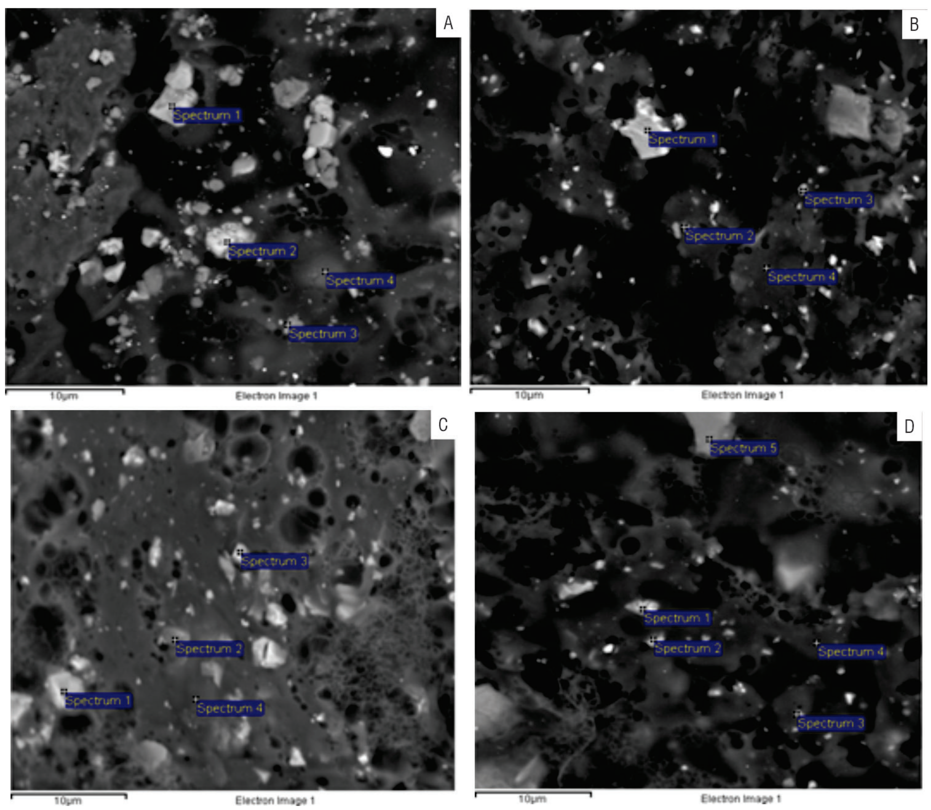

Fig 2: SEM of chewing gum after chewing experiment. (A) 2.5% BAG low BG (B) 5% BAG low GB, (C) 2.5% BAG high GB, (D) 5% BAG high GB

TABLE 2: 2.5% BAG LOW GB SEM/EDS ANALYSIS BEFORE CHEWING (ALL RESULTS IN WEIGHT%)

Spectrum	C	O	F	Na	Si	P	Ca	Total
Spectrum 1	37.19	38.21	-	-	-	-	24.60	100.00
Spectrum 2	67.35	17.49	1.36	1.55	5.32	2.34	4.58	100.00

TABLE 3: 5% BAG LOW GB SEM/EDS BEFORE CHEWING (ALL RESULTS IN WEIGHT%)

Spectrum	C	O	Na	Mg	Al	Si	Ca	Total
Spectrum 1	39.20	32.13	-	-	-	-	28.67	100.00
Spectrum 2	50.65	25.34	-	-	-	-	24.01	100.00
Spectrum 3	38.62	40.52	-	-	-	-	20.86	100.00
Spectrum 4	77.22	13.41	0.31	2.12	0.84	2.42	3.69	100.00

TABLE 4: 2.5% BAG HIGH GB SEM/EDS ANALYSIS BEFORE CHEWING (ALL RESULTS IN WEIGHT%)

Spectrum	C	O	S	Ca	Total
Spectrum 1	-	-	-	100.00	100.00
Spectrum 2	-	5.62	-	94.38	100.00
Spectrum 3	71.83	7.22	-	20.95	100.00
Spectrum 4	52.36	4.51	-0.06	43.19	100.00
Spectrum 5	74.46	13.18	-	12.36	100.00
Spectrum 6	73.81	9.82	-	16.37	100.00

TABLE 5: 5% BAG HIGH GB SEM/EDS ANALYSIS BEFORE CHEWING (ALL RESULTS IN WEIGHT %)

Spectrum	C	O	Na	Si	P	Ca	Total
Spectrum 1	31.77	43.71	-	24.52	-	-	100.00
Spectrum 2	37.68	34.60	-	-	-	27.71	100.00
Spectrum 3	56.72	23.91	1.03	7.70	2.84	7.80	100.00
Spectrum 4	82.05	17.95	-	-	-	-	100.00
Spectrum 5	22.44	35.87	-	-	-	41.69	100.00

TABLE 6: 2.5% BAG LOW BG SEM/EDS AFTER CHEWING (ALL RESULTS IN WEIGHT %)

Spectrum	C	O	F	Na	Mg	Al	Si	P	Ca	Total
Spectrum 1	39.41	21.13	-	-	0.30	-	0.32	-	38.84	100.00
Spectrum 2	22.85	39.18	2.52	3.51	-	-	12.01	4.03	15.89	100.00
Spectrum 3	46.94	36.95	-	0.15	-	14.83	0.18	-	0.94	100.00
Spectrum 4	77.30	14.99	-	0.31	0.58	-	1.08	-	5.75	100.00

TABLE 7: 5% BAG LOW GB SEM/EDS AFTER CHEWING (ALL RESULTS IN WEIGHT%)

Spectrum	C	O	Na	Mg	Si	Ca	Total
Spectrum 1	24.84	49.25	3.95	-	-	21.97	100.00
Spectrum 2	67.46	19.24	4.54	3.36	5.39	-	100.00
Spectrum 3	31.55	48.08	0.25	-	-	20.12	100.00
Spectrum 4	80.39	9.84	2.77	2.59	4.41	-	100.00

TABLE 8: 2.5% BAG HIGH GB SEM/EDS AFTER CHEWING (ALL RESULTS IN WEIGHT%)

Spectrum	C	O	Mg	Si	Ca	Total
Spectrum 1	36.63	23.42	-	-	39.96	100.00
Spectrum 2	73.40	13.88	1.27	-	11.45	100.00
Spectrum 3	78.76	14.00	-	0.78	6.45	100.00
Spectrum 4	91.14	4.53	0.55	1.02	2.75	100.00

TABLE 9: 5% BAG HIGH GB SEM/EDS AFTER CHEWING (ALL RESULTS IN WEIGHT %)

Spectrum	C	O	Na	Mg	Al	Si	P	Ca	Total
Spectrum 1	28.10	27.74	-	-	-	-	-	44.16	100.00
Spectrum 2	52.22	7.34	0.10	0.39	0.34	0.76	0.25	38.59	100.00
Spectrum 3	92.38	6.60	-	-	-	-	-	1.02	100.00
Spectrum 4	95.49	4.51	-	-	-	-	-	-	100.00
Spectrum 5	69.24	18.63	0.44	2.74	-	6.85	-	2.11	100.00

chewing to analyze the remaining of the glass particles and of calcium carbonate in GB (figure 2, tables 6-9). The results show that some of these glass particles and calcium carbonate remain there along with talc or mica. The SEM/EDS analysis of 2.5% BAG low GB spectrum 2 shows the elements of both glass and calcium carbonate whereas spectrum 3 identifies elements present in mica along with calcium carbonate (figure 2, table 6). 5% BAG low GB spectrum 3 identifies the elements of glass and mica (table 7). 2.5% and 5% BAG high GB spectrum1 and 2 identify carbon, oxygen, and calcium (table 9). Bakry et al.[11] investigated BioMinF® paste chemical composition by using SEM/EDS for calcium, phosphorus, carbon, oxygen, fluoride, oxygen, and silicon to examine the potential of the BiominF® paste as a remineralizing agent. SEM/EDS examination showed calcium and phosphate ions catalyzation in the presence of fluoride ions, thus forming acidic calcium-phosphate salts layer on demineralized enamel surface.[10,11,15-18] With the help of EDS analysis Siqueira and Zanotto[19] found the surface layer compositions of the BAG and observed noticeable increase in the concentration of calcium and phosphate compared to silica. In another study EDS analysis showed a noticeable decrease in silica on the surface of glass-ceramics and increase in calcium and phosphate concentration which can result in formation of new calcium and phosphate rich layer on the sample surface.[20] In this study, presence of calcium and phosphate in chewing gum containing BAG might be indicative of calcium and phosphate release during chewing process that may have been utilized in formation of apatite layer.[21] De Caluwé *et al*. observed crystalline structure on the surface of 45S5F type BAG along with traces of different elements used in BAG similar to this study.[22]

Hence the results of this study showed the constituents and random distribution of BAG particles in chewing gum base before and after chewing. Moreover, tris solution in which gum is chewed might have ions released by BAG. This solution can be further examined for the fluoride, calcium, and phosphate levels to confirm the FAP formation and to detect the exact role of these ions concentration in the BAG containing chewing gum. A limitation of this study could be that manual chewing experiment could not be standardized fully hence may result in biases. Although all the experiments of manual chewing with fingers were performed by single person, further clinical trials may be more useful in this regard.

CONCLUSION

The BAG particles are randomly distributed in chewing gum base. Moreover, BAG containing calcium, phosphate, and fluoride in chewing gum base can have potential for enamel remineralization. Further clinical studies are required for evaluation along with refinement.

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3	**Um-e-Rubab Shirazi:**	Plagiarism check, proof reading
4	**Erum Minhas:**	Expert opinion and experience in finalizing the manuscript