SEM Analysis of the Cellular Bio-hospitality and Roughness of Glass Dental Ceramics

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Abstract

Biocompatibility of dental materials is dependent on the composition, pretreatment, and handling of the materials. The variety of ceramic restoring materials is based mainly on different composition, strength and fracture toughness more than biocompatibility. This study evaluated the surface roughness and cellular proliferating rate of feldspathic (duceram) and hydrothermal (duceragold) ceramics for dental restorations. Twenty square samples of 1 cm^2 for each group of ceramic, prepared according to the manufacturers guidelines were used in the present study. Quantitative change in surface roughness were analyzed with profilometer before cell incubation. To evaluate the cellular proliferation rate, A-431cells derived from human epidermis carcinoma cell line cultivated in modified synthetic fetal serum were used. After 24- hour of incubation the ceramic plates were washed, pre-fixed with buffered glutaraldehyde and paraformaldehyde at 2.5% and post-fixed in a 2% osmium tetroxide solution. The specimens, after dehydration , CPD and gold metallization, were analyzed with SEM and the surface areas covered by cells were measured with an image analysis software (Sigma Scan Pro5, SPSS Science, Chicago, USA). The results were statistically evaluated using z- test and t-test at a significance level of $P<.05$. The area covered by cells measured in square pixels was 40,30 % for duceram and 77,14 % for duceragold. The difference was statistically significant (z-test, P< .05). The roughness analysis showed an Ra value of 0.097±0.029 m (mean ± SD), for duceragold and 0.658±0.387 m (mean±SD) for duceram, with a difference statistically significant (t-test, P< .05). The 3D reconstruction of the sample's surfaces based on SEM images showed microscopic defects on all samples. The results of this investigation showed that Hydrothermal ceramic has a surface more smooth and bio-hospital than conventional feldspathic ceramic.

Introduction.

A dental material that is to be used in the oral cavity should be biocompatible otherwise it must be able to be in contact with living tissue not causing toxic or injurious effects [1]. The oral environment is especially hostile for dental restorative materials, bacteria are ever present and saliva has corrosive properties. This environment demands appropriate biological tests and standards for evaluating any material that is developed and intended to be used in the mouth [2]. In designing restorative materials, dental scientists give particular attention to several key factors relating to a material's biocompatibility with the human organism. These include potential tissue responses, leakage of bacteria at the tooth-filling interface, shrinkage of materials, and stress created in the tooth structure from restoration's procedures[2]. The Council on Dental Materials, Instrument, and Equipment of the American National Standard Institute/ American Dental Association (ANSI/ADA) for biological evaluation of dental materials commanded the

use of primary tests (to indicate cellular response), secondary tests (to evaluating tissue responses), and usage tests in animals before being evaluated clinically in humans. However, the clinical significance of these tests is unsettled and there is poor correlation between the results of different tests [3,4]. The relative incidence of biological side effects of dental ceramics compared with other restorative materials is considered to be low. In general, conventional dental ceramics are considered to be the most inert of all materials used for dental restorations. Ceramic restorative materials are not known to cause biological reactions, except for wear on the opposing dentition and/or restorations [5]. No long-term data on the biocompatibility of these restorations are available. Special attention should be paid to the assessment of ceramic materials which come in contact with periodontal tissues as in the pontic areas of the fixed prosthodontics. The morphological relationship between prosthetic materials and epithelial cells is an important topic since it is affected by several factors as composition, micro/macro-morphology and wetting features. Most of the ceramic materials available on the market are feldspatic materials with dispersed crystalline phase in the glass matrix (Fig. 1).

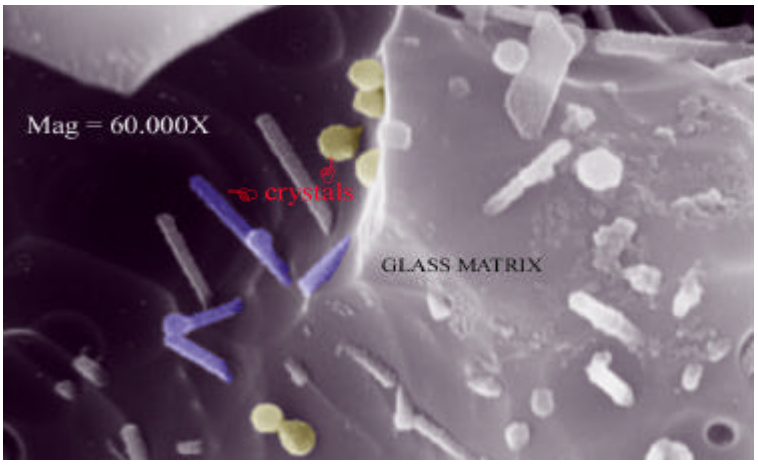

Figure 1. Micrograph of feldspatic ceramic. The crystals and the glass phases are visible.

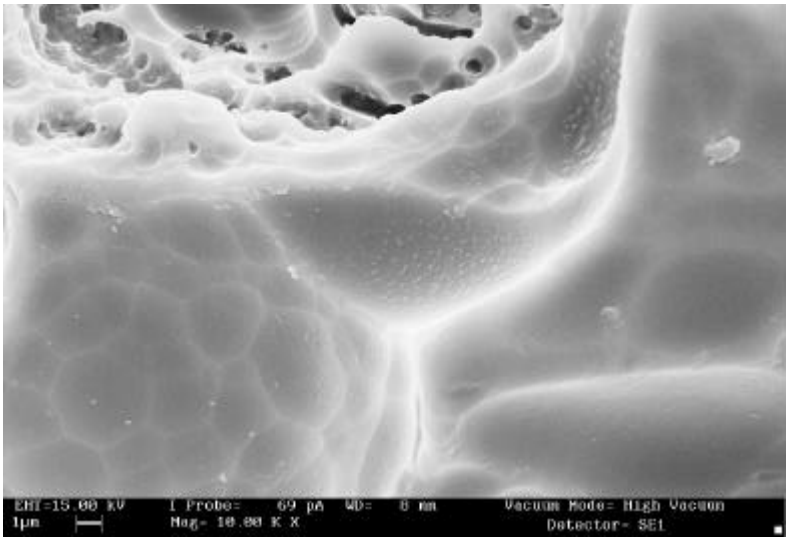

Figure 2. Micrograph of glass ceramic. The generous glass phase is visible.

The recent introduction of a new dental ceramic material i.e. the “hydrothermal ceramic” (Fig. 2)

with innovative chemical and physical properties is particularly interesting [6]. In this material the quartz lattice, which forms the vitreous phase, is modified by adding alkali oxides, which lower the melting temperature (approximately 800 °C) increasing the coefficient of expansion. The presence of a one micron of hydroxide layer (film) on the material surface seals the micro-fractures, and reduces the detriment and roughness [7,8,9] while the presence of the hydroxides in the silicate lattice affects the entropy and enthalpy level which is connected to the variation of the OH groups. The lowering of the surface energy, thus potentially change the biological reaction of the ceramic material [10]. In the 1996 Barclay CW et al. highlighted the LFC ceramic capability to generate a mucous attack, similar to the dental – gingival one, when used as abutment material [11]. This paper aims at evaluating the relationship between living cells proliferation rate and surface roughness of both hydrothermal and traditional feldspathic ceramic materials.

Materials and methods

Two different ceramic materials were used for this study: duceragold and duceram (Ducera Dental GmbH, Rosbach, Germany). All the samples were prepared by the same skilled operator, according to the manufacturer's guidelines. 20 samples per group, i.e. 5 enamel and 5 dentine samples, composed of 1 cm^2 plates, were prepared. All the samples were cleaned with a 180 °C saturated steam jet and then accurately washed in a 95% ethyl alcohol solution for 15 minutes, in an ultrasound bath (250W) heated at 40°C. The surface roughness were measured for all samples using a profilometer Hommel Tester T8000 (Hommelwerke GmbH, Schwenningen, Germany) , than five samples randomly selected from each group were analysed by means of a SEM (LEO 435 Vp Cambridge, UK). Following the surface analysis, all the samples were marked ,cleaned and washed again. Finally they were sterilised twice, consecutively, in a fractional vacuum autoclave Easy R (Faro Spa, Ornago, Milano, Italy) at 134° C for 20 minutes.

Cellular culture

The ceramic plates were incubated with human epidermis carcinoma cells (A-431) cultivated in modified synthetic "Eagle" (DME) with fetal serum, as per Dulbecco's method. After a 24-hour incubation, the plates were washed in 0.1M phosphate buffer with 0.15M of sucrose to ensure the osmolarity remained at about 360 mOsm, prefixed for 20 h at 4° C in a 5 ml of glutaraldehyde at 2% in 0.05M phosphate buffer (pH 7.4) and after washed again with buffer solution. Following prefixation the specimens were treated with OTOTO method of post fixation as Malik-Wilson involving repeated exposure to osmium tetroxide and thiocarbohydrazide[12,13]. All specimens were than washed Six times in distilled water for a total of 15 minutes, dehydrated using a series of graded alcohol and Critical Point Dried (CPD) from liquid CO2 in an Emitech K 850 (Emitech Ltd. Ashford, Kent,UK). All samples were mounted onto aluminium specimens holders using carbon adhesive discs, lightly coated with gold in an Emitech K 550 sputter coater (Emitech Ltd. Ashford, Kent,UK).

SEM observation and image analysis

The samples were examined and photographed using SEM (LEO 435Vp Cambridge, UK) by detecting Secondary Electrons (SE1 mode) accelerated to an energy of 15-20 keV.

The quantitative analysis was carried out using the SigmaScan Pro5 software (SPSS Science, Chicago, USA) on detected SEM images (TIF-format) of 1024x768 pixels with a resolution of 100x100 pixels at 8 bit. The surface texture were 3D reconstructed by the Scanning Probe Image Processor (SPIP) for Windows Version 2.3037(Image Metrology, Lyngby, Danmark)

Statistical analysis

The surface roughness Ra was analysed using t test with a power of 0.994 at á = 0.050. The cellular proliferation rate were evaluated by comparing proportion z-test with Yates correction. The power of test was 0.535 at á = 0.050.

Results

The SEM surface analysis showed a slight difference with reference to the surface finish of the two ceramic groups (Figs. 3,4).

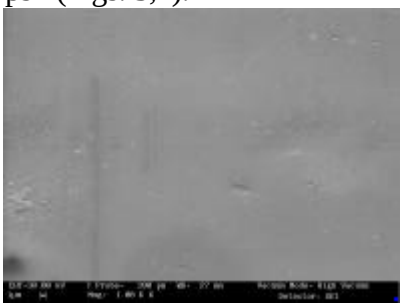

Figure 3. SEM image of duceragold surface.

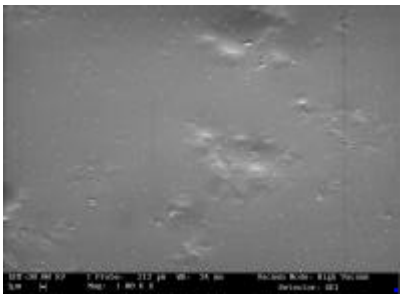

Figure 4. SEM image of duceram surface.

Nevertheless microscopic defects were found on all samples. The Ra values were for duceram 0.658±0.387 .m (mean±SD) while for duceragold 0.097±0.029 .m (mean ± SD) (Fig. 5,6).

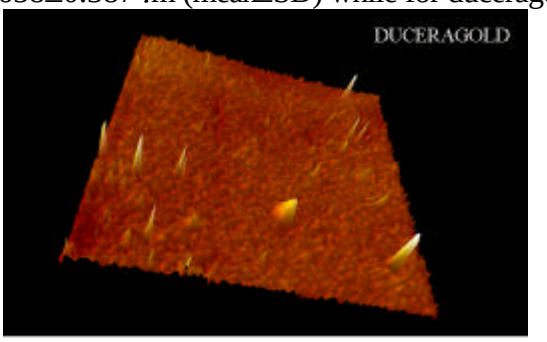

Figure 5. 3D reconstruction of Fig. 3 SEM image.

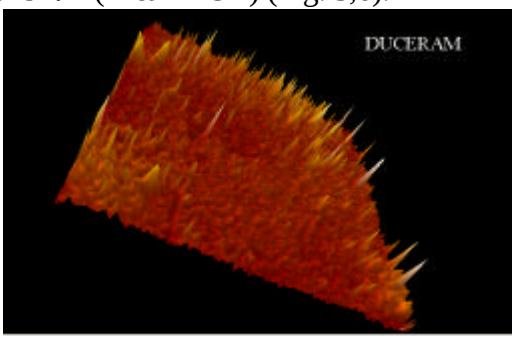

Figure 6. 3D reconstruction of Fig.4 SEM image.

The surfaces roughness of the ceramics were significantly different (P=0.00) at t test (Table1).

Table 1

t-test comparison of the surface roughness

	N of cases	Mean [ì m]	SD	SEM
Ra duceram*	20	0.658	0.387	0.08654
Ra duceragold*	20	0.097	0.029	0.006485
Mean Difference		0.561		0.08678
95% CI of difference	From 0.3853 to 0.7367			
t	6.465			
DF	38			
P	0.000			

*statistically significant

The cellular adhesion analyses (Figs. 7, 8, 9) showed that, as far as duceram is concerned, out of a surface of370239.8 ± 75396.84 (mean ± SD) square pixels, 149206.6±24592.52 were covered by cells, i.e. approximately 40.30% of the total surface (Fig. 10), while, duceragold, 288687.9±27143.31 square pixels out of 374239.0±28760.9, i.e. 77.14% of the total surface, were covered by cells (Fig. 11).

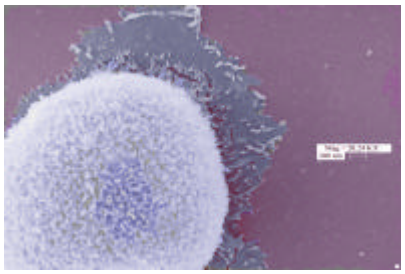

Figure 7. A-431 cell on duceragold .

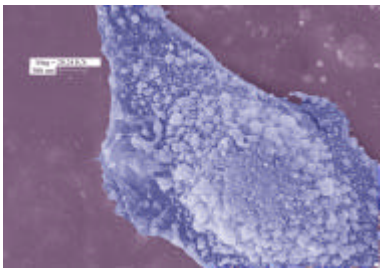

Figure 8. A-431 cell on duceram.

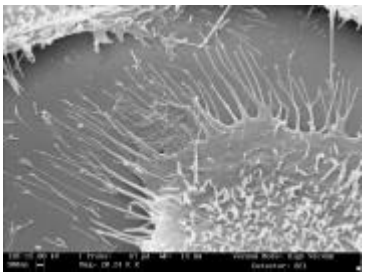

Figure 9. A-431cells on glass cover slip.

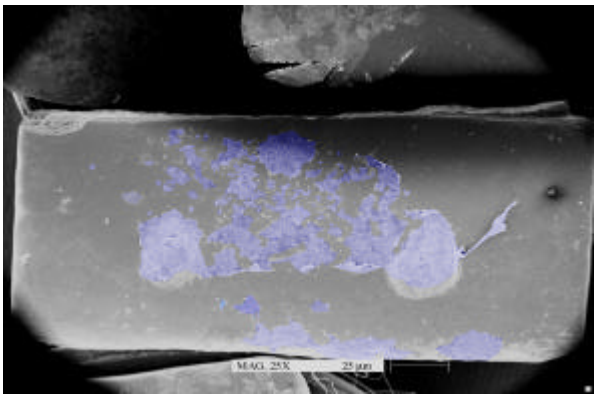

Figure 10. SEM image of duceram specimen. The pseudo-coloured area is covered by cells.

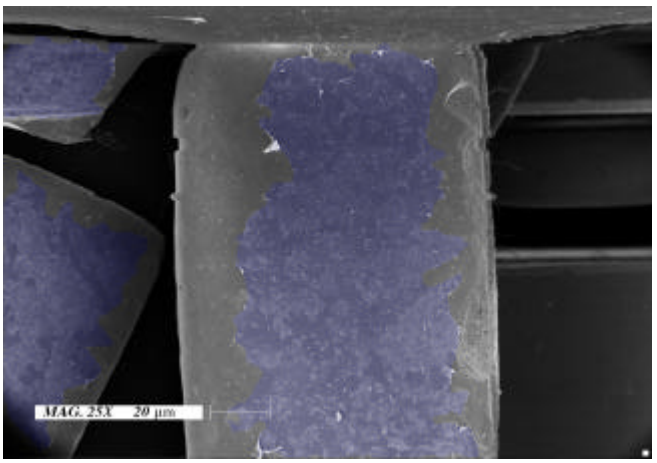

Figure 10. SEM image of duceragold specimen. The pseudo-coloured area is covered by cells.

The z-test statistical analysis for comparing proportions showed a significant difference between cellular bio-hospitality in the two groups of ceramics (P=0.041) (Table 2).

Table 2

z-test comparing proportion of the cellular proliferating rate referred as % of the total mean area of the specimens covered by cells [square pixels]

	N of cases	Area %	Difference of sample proportions	Pooled estimate for p=
Duceragold*	20	0.403	-0.368	0.587
Duceram*	20	0.771		
Standard error of difference of sample proportions	0.156			
95% CI for difference	-0.673			
z	2.042			
p	0.041			

*statistically significant

Discussion

Many clinicians have advocated glazed porcelain as the preferred or only material that should touch the edentulous ridge. Other underlined that the proper design of the pontic is more important to cleanability and good tissue health than is the choice of materials. The long-term success criteria for ceramic restoration depends on many factors such as the technicians' manual skills, the quality of the materials used and the operators' know-how.

Nevertheless the our results shows a relation between composition of material and cell colonization both in quality and quantity. The introduction of hydroxyl groups in the glass, (Eq. 1)

$$\equiv Si - O - Si \equiv + H_2O \rightarrow 2 \equiv Si - OH \quad (1)$$

dating back to the sixties [14,15]. Nevertheless the process was further improved and controlled in the Eighties through the overheating of melted glass in an atmosphere saturated with hydroxide ions steam [16,7]. The hydroxyl groups substitutes the alkali present on ceramic surface forming a plastic layer (Eq. 2)

$$\equiv Si - O - Na^+ + H_2O \rightarrow 2 \equiv Si - OH + Na^+ + OH^- \quad (2)$$

these groups increases the strength and change the surface characteristics of the ceramic[8].This is the foundations of the new pyrocerams, i.e. materials with a very smooth surface. The highly-magnified SEM image of duceragold samples shows that after 24 hour of incubation the membrane of A-431 cells express several filopodes (also referred to as lamellipods or microvilli) as is usually in an active spreading process. The major expression of filopodes was noted on membrane side towards cell-free areas of ceramic surface while they were inhibited towards areas covered by cells suggesting a form of cellular "vision" [17] (Figs. 12,13).

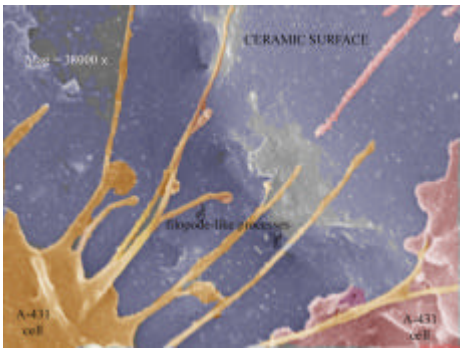

Figure 12. SEM image of duceragold samples.

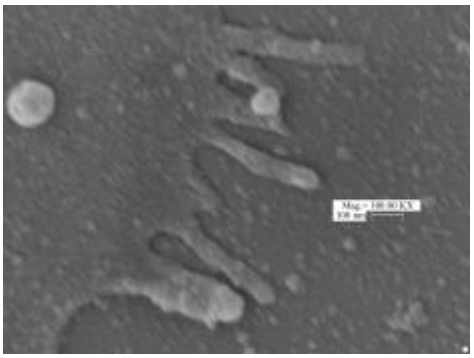

Figure 13. SEM image at limit of resolution of duceragold samples. Microvilli are visible.

The cytoplasmic membrane of the cells was strongly adhered to the ceramic, as much as it remained attached to the surface overcoming the fracture of cell body (Fig. 14).

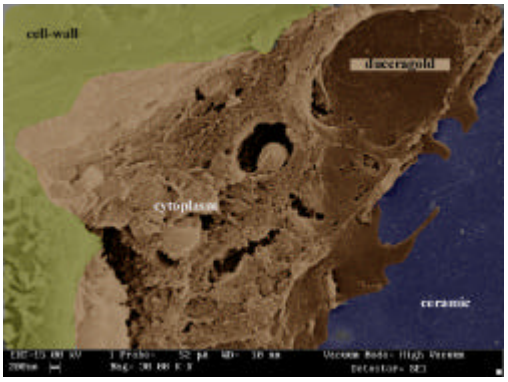

Figure 14. SEM image of duceragold samples with A-431 cell after cell body fracture.

These could be related to proteins that bind to inorganic surfaces with high binding energy by chemisorption [18] . The protein adsorption on ceramic surface is influenced by interfacial tension, solubility and the electrical charge of the surface. The chemical-electrical interaction may play a more important role with reference to the surface roughness, as far as the cellular adhesion is concerned (Fig. 15).

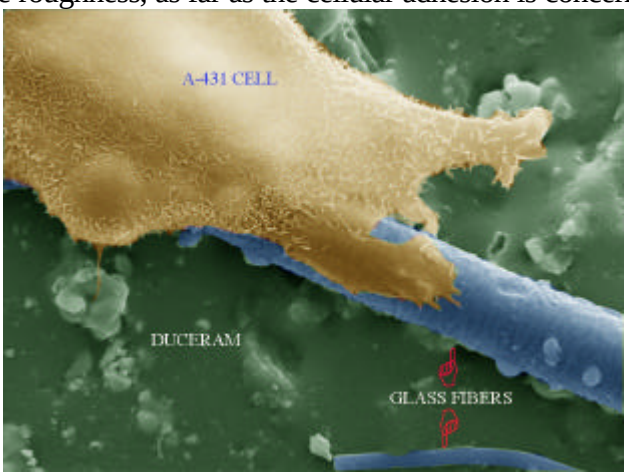

Figure 15. SEM image of duceram samples. In blue two glass fibres “attached” to the ceramic surface that belongs to the firing support. The only one cells present on this area is strongly attached to them.

The surface roughness of the ceramic materials is a key factor of long lasting dental restorations because it directly influence the clenability and the microbes retention. In the oral cavity there are several species of

microbes that mostly are associated to the surface's of the oral structures (also dental prosthesis) as dental plaque a multiple-species biofilm [19]. This is the prevailing microbial life-style in the oral cavity because surface association is an efficient means of lingering in a favourable microenvironment rather than being swept away by the saliva. The high level of bio-hospitality of a material used for prosthetic appliances should not be useful in terms of biofilm formation, nevertheless this characteristic if is associated to a smooth and shine surface there is not a disadvantage. The shine and smooth surfaces facilitating the removal of dental plaque by toothbrush.

Conclusion

The results of this study indicated that there is the significant differences between the two ceramic. Duceragold has excellent features in terms of bio-integration/bio-inertia because the proliferation rate and the surface roughness are more favourable than in the duceram.

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