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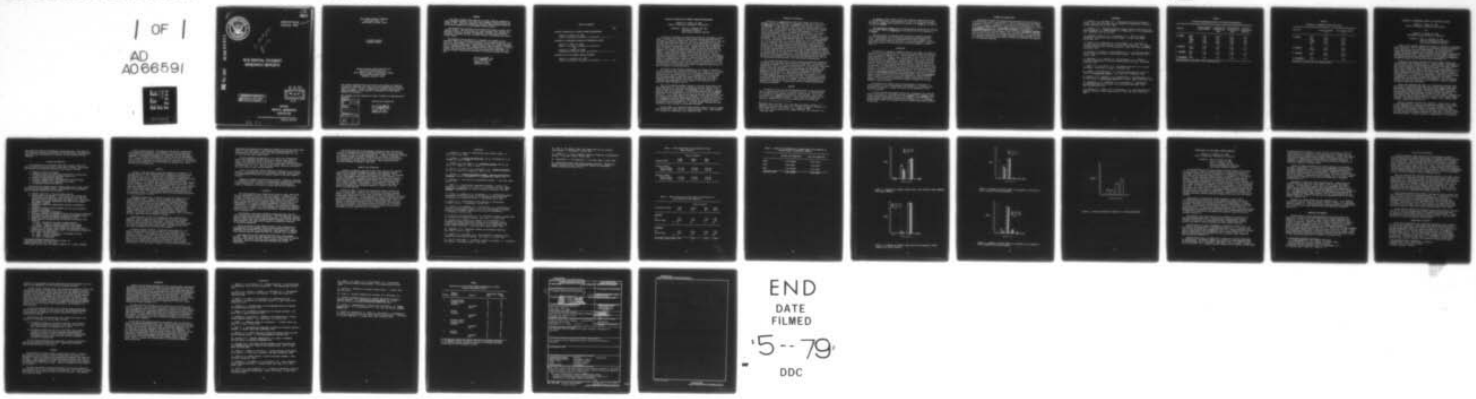

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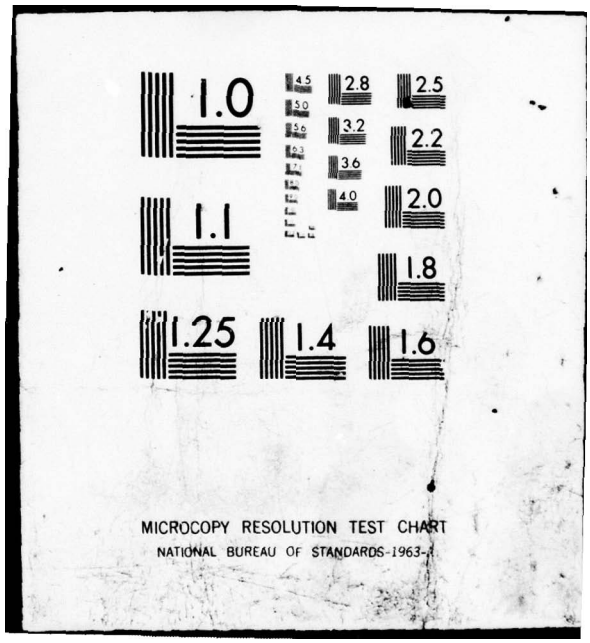
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Naval Medical Research and Development Command
Bethesda, Maryland

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1978 DENTAL STUDENT RESEARCH REPORTS

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Approved and released by:

M. R. Wirthlin, Jr.

M. R. WIRTHLIN, JR.
Captain, DC, USN
Commanding Officer

PREFACE

This report includes three reports on original research conducted by Dental Students assigned to the Naval Dental Research Institute during the summer of 1978. They were assigned by the Naval Health Sciences Education and Training Command for the 1978 Summer Training Program.

The students were provided a list of topics from which to select their research project. With the guidance of their preceptor they reviewed the pertinent literature, designed the experiment, performed the laboratory procedures, collected the data, analyzed the data, and prepared the initial written report.

The purpose of the projects was to introduce the students to the scientific method and concurrently contribute to the research mission of the Institute. Each student, in his individual research area, became knowledgeable in hypothesis development, research design, sample selection, variability control, instrumentation, data interpretation and logical reasoning. It is evident from the following reports that the purpose was accomplished, and that the students experienced a meaningful and productive period of active duty.

M. R. WIRTHLIN, JR.
Captain, DC, USN
Commanding Officer

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FLUORIDE ACCUMULATION BY DENTAL PLAQUE MICROORGANISMS

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The ability of plaque to concentrate fluoride has been known for many years. Hardwick first reported a mean plaque fluoride concentration of 66.9 ppm in a study of a population living in a fluoridated area in 1962 (1). He later reported concentrations of 32.2 and 42 ppm fluoride in similar populations in 1970 (2). Until recently this fluoride was not believed to have any potential therapeutic value. Prophylaxis has been recommended prior to topical fluoride application, based on studies noting that the omission of a cleaning procedure prevented fluoride penetration into the enamel (3) and reduced the caries inhibition effect by up to 50% (4). However, current research suggests that plaque may serve as a source of fluoride incorporated into enamel and that the post-eruptive, environmental effects of fluoride may be related to these high fluoride levels in plaque. Studies have demonstrated a preparation of the enamel surface for fluoride uptake by plaque acids (5), and a direct correlation between the fluoride concentration of surface enamel and the overlying plaque volume (6). This work has lead to new interest in the nature of plaque fluoride and how it may be utilized in caries prevention.

The source of the fluoride accumulated in the plaque is believed to be oral fluids and both liquid and solid foods (7). However, since parotid saliva was found to contain only 0.01 to 0.29 ppm fluoride after direct analysis with a fluoride electrode (7), the absorption of the fluoride must be accomplished by other than simple diffusion into the plaque. Concentration of the fluoride may occur in the bacterial cell wall or cytoplasm, the plaque matrix, or by binding of the fluoride to low molecular weight inorganic ions (8).

Many of the factors possibly involved in fluoride accumulations have been investigated (7), but little information has been reported on the actual ability of the different microorganisms found in plaque to take up fluoride from dilute solutions. In 1976 Kashket reported that S. sanguis H7PR3 was able to concentrate the ion up to 8 fold over the surrounding medium (8). In the same paper it was reported that the fluoride entry into the cells was very rapid, peaking at 30 minutes. The uptake of fluoride by S. mutans 6715 has also been examined and shown to be related to the pH of the fluoride containing media. The ability to concentrate fluoride increased as the pH decreased (8,9). No other plaque organisms have been examined for their ability to concentrate fluoride.

In this report, ten different microorganisms, known to exist in plaque, were evaluated for their ability to take up fluoride from a 1 ppm solution at a constant pH, temperature, and incubation time.

METHODS AND MATERIALS

Cultures of S. mutans (strains HS-6, BHT, 10449, 6715, LM-7), S. sanguis (strains 167 and 10558), A. viscosus (strains 626 and 1113) and A. naeslundii (strain 398A) were tested for their ability to take up fluoride from a 1 ppm solution. Each culture was grown 24 hours in Todd-Hewitt broth. These cultures were centrifuged at 4,000 RPM for 5 minutes and the broth was discarded. The pellet was suspended in 1 ml of 1 M sodium acetate buffer, pH 5.5, transferred to preweighed 1.5 ml plastic centrifuge tubes[a] and centrifuged at 15,000 RPM for 1.5 minutes in a microcentrifuge.[b] The buffer was discarded and the wet weight of the cells determined. Yields were approximately 28-32 mg. The cells were incubated at 37°C for 30 minutes in 80 µl of the buffer, 10 µl of 0.1 M glucose and 10 µl of 10 ppm fluoride, for a final concentration of 1 ppm fluoride and 0.01 M glucose. Two identical solutions without cells were used as controls. The supernatant solution was removed after centrifugation at 15,000 RPM for 1.5 minutes and read with a fluoride ion specific electrode.[c] The fluoride concentration within the cells was calculated using the amount of fluoride taken up and the intracellular water volume, estimated to be 70% of the wet weight of the pellet (4). The extracellular fluoride concentration was taken as the final concentration of the supernatant solution, and the ability of the organisms to take up fluoride was expressed as the ratio of the intracellular concentration to the extracellular concentration.

All of the fluoride taken up by the various microorganisms tested was recoverable from the pellet after removing the supernate. The surface of the pellet was washed with 1 ml of deionized water. The wash was lyophilized, redissolved in 100 µl of TISAB[d] and then read for fluoride content. The amount of fluoride remaining in the microorganisms was determined using a modification of the microdiffusion process as described by Hallsworth, Weatherell and Deutsch (10). Each pellet was digested in 200 µl 70% perchloric acid at 60°C. The liberated fluoride was collected in 5 µl of 1 M NaOH suspended from the lid of the test tube. The amount of fluoride recovered was determined with the fluoride electrode after dissolving the dried NaOH in the lid in 100 µl of TISAB. With each microdiffusion run, separate microdiffusions of appropriate amounts of sodium fluoride were included as standards.

RESULTS

The ability of the various microorganisms tested to take up fluoride from a 1 ppm solution of the ion is shown in Table 1. The results shown are the means of four separate cultures of each organism. Four of the five strains of S. mutans concentrated the fluoride about three-fold over the level of the surrounding medium. Strain 10449 was somewhat less effective, demonstrating a two-fold concentration of the fluoride.

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[a] Eppendorf Micro Test Tube, 1.5 ml, Brinkman Instruments, Westbury, N. Y.
[b] Eppendorf Microcentrifuge, Model 5412, Brinkman Instruments, Westbury, N. Y.
[c] Orion Model 96-09, Orion Research, Inc., Cambridge, MASS.
[d] Total Ionic Strength Buffer, Orion Number 94-09-09, Orion Research, Inc., Cambridge, MASS.

S. sanguis, strain 10558, was the most effective organism tested with an internal concentration approximately 4.5 times the extracellular level. The other S. sanguis strain examined was similar to S. mutans in its ability to take up fluoride.

The Actinomyces viscosus strains examined showed essentially no fluoride concentration, while A. naeslundii took up amounts of the ion similar to S. mutans.

The total amount of fluoride taken up from the incubation mixtures could be recovered in the cell wash and perchloric acid extraction of cells. Approximately 30% of the absorbed fluoride was released in the water wash, and the remainder was found in the microorganisms on digestion. The fluoride taken up and recovered for each strain examined is shown in Table II.

DISCUSSION

The fluoride in plaque is thought to exist in two forms. The first is as the free ion in plaque fluid; the second is bound by bacteria, epithelial cells, or inorganic components (7). The concentration of the free ion has been reported to be 0.5 ppm or less, and to make up only 2-5% of the total plaque fluoride (7). Likewise, it is doubtful that much, if any, fluoride is taken up by the plaque matrix. Jenkins, Edgar, and Ferguson found that when the matrix was isolated and dialyzed against a 1 ppm sodium fluoride solution, the fluoride concentration within the matrix was the same as that of the media (11), demonstrating a lack of fluoride binding by this cell-free material. The results of this study indicate that the fluoride binding may be due to the action of the microorganisms alone. All of the microorganisms except the Actinomyces viscosus strains concentrated fluoride two- to four-fold over the concentration in the surrounding media. Even the Actinomyces uptake, although low, was above the level expected for simple diffusion. Similar levels of binding by organisms in plaque could account for the amounts of fluoride found there when the plaque is assayed using similar methods to those used here.

The results also indicate that the concentration of fluoride is a common property of oral microorganisms, and therefore it is doubtful that the levels of fluoride in plaque are related to the presence of a few selected organisms.

In the preliminary experiments carried out to determine the conditions used in this study, we also found that the S. mutans and S. sanguis strains tested followed similar patterns of rate of uptake as noted by Kashket (8), and pH effects as described by Pashley and co-workers (9). All organisms showed maximum uptake by 30 minutes, and had increased uptake when incubated at lower pH.

SUMMARY AND CONCLUSIONS

Ten strains of common oral bacteria were evaluated for their ability to take up fluoride from a 1 ppm solution in order to determine if this property is present in most oral organisms or in a few selected organisms only. Five strains of S. mutans, comprising a representative of each serotype, two strains of S. sanguis, two strains of Actinomyces viscosus and one strain of Actinomyces naeslundii were tested. The two strains of Actinomyces viscosus showed essentially no fluoride uptake, while the other organisms concentrated the ion 2 to 4.5 fold over the extracellular concentration. It was concluded that fluoride concentration is a common property for these organisms and thus it is doubtful that the fluoride levels in plaque are related to the presence of a few selected organisms.

REFERENCES

1. Hardwick, J. L. and Leach, S. A. The Fluoride Content of the Dental Plaque. Proc. 95th Cong. Eur. Org. for Research on Fluoridation and Dental Caries Prevention, pp. 151-158, 1962.

2. Hardwick, J. L. Associations between plaque fluoride concentrations and other parameters. In: DENTAL PLAQUE, W. D. McHugh (ed.), E. & S. Livingstone Ltd., Edinburgh 1970, pp. 171-178.

3. Tinanoff, N., Wei, S. H. Y., and Parkins, F. M. Effect of pumice prophylaxis on fluoride uptake on tooth enamel. J. Amer. Dent. Assoc. 88:384-389, 1974.

4. Knutson, J. W., Armstrong, W. B., and Feldman, F. M. The effect of topically applied sodium fluoride on dental caries experience. IV. Report of finding with two, four and six application. Publ. Hlth. Rep., Washington, 62:425-530, 1947.

5. Charlton, G., Blainer, B., and Schamschula, R. G. Associations between dental plaque and fluoride in human surface enamel. Arch. Oral Biol. 19: 139-143, 1974.

6. Weatherell, J. A., Robinson, C., and Hallsworth, A. S. Changes in fluoride concentrations of the labial enamel surface with age. Caries Res. 6:312-324, 1972.

7. Edgar, W. M. and Jenkins, G. N. Distribution and forms of F in saliva and plaque. Caries Res. 11 (Suppl. 1):226-242, 1977.

8. Kashket, S. and Rodriquiz, V. M. Fluoride accumulation by a strain of human oral Streptococcus sanguis. Arch. Oral Biol. 21:459-464, 1976.

9. Pashley, D. H., Schuster, G. S., Venkaleswarler, P. and Whetford, G. M. Fluoride uptake by Streptococcus mutans 6715. Infect. Immun. 18:680-687, 1977.

10. Hallsworth, A. S., Weatherell, J. A. and Deutsch, D. Determination of subnanogram amounts of fluoride with the fluoride electrode. Anal. Chem. 48:1660-1664, 1976.

11. Jenkins, G. H., Edgar, W. M., and Ferguson, D. B. The distribution and metabolic effects of human plaque fluorine. Arch. Oral Biol. 14:105-119, 1969.

TABLE I

FLUORIDE CONCENTRATING ABILITY OF PLAQUE MICROORGANISMS

Organism		Fluoride Uptake* (ng/mg plaque)	Intracellular* Concentration (ppm)	Extracellular* Concentration (ppm)	Fluoride Concentrating Ability
S. mutans	HS-6	1.43	2.04	0.72	2.8
	BHT	1.63	2.32	0.74	3.1
	10449	1.44	2.05	0.91	2.2
	6715	1.88	2.68	0.92	2.9
	LM-7	1.99	2.84	1.00	2.8
S. sanguis	167	1.86	2.65	0.97	2.7
	10558	2.67	3.81	0.84	4.5
A. viscosus	1113	1.00	1.42	1.04	1.3
	626	1.22	1.74	1.02	1.7
A. naeslundii	398A	1.65	2.35	0.94	2.5

*Values shown are the means of four determinations

TABLE II

RECOVERY OF ABSORBED FLUORIDE FROM CELLS

Organisms		Fluoride Uptake* (ng)	Fluoride Recovered* (ng)
S. mutans	HS-6	59.8	61.8
	BHT	54.8	53.0
	10449	40.5	47.9
	6715	34.8	65.8
	LM-7	47.2	66.4
S. sanguis	167	52.8	59.6
	10558	63.8	59.0
A. viscosus	1113	28.5	63.8
	626	46.0	46.1
A. naeslundii	398A	54.0	37.2

*Values are the means of four determinations

INFLUENCE OF INTERDENTAL CONTACTS ON PERIODONTAL STATUS

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and

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Loose or open proximal contacts are thought to contribute to periodontal pocket formation. Lack of integrity of the proximal contact is considered with other factors such as calculus deposits, ill fitting margins of dental restorations, proximal carious lesions, food impaction, and plunger cusp relationships to be a secondary etiologic agent in inflammatory periodontal disease (1-6). However, the role of the proximal contact in the maintenance of periodontal health and in the etiology of inflammatory periodontal disease has not been definitely established (7).

Reports by some authors indicate that tight proximal contacts may not be necessary for periodontal health. O'Leary, Baudell, and Bloomer (8) reported that a group of periodontally healthy young male dental students had a high percentage of open or defective contacts. In a study of dried skulls, Larato (9) found no significant relationships between factors such as open or defective contacts and interproximal intrabony lesions. Geiger, Wasserman, and Turgeon (10) reported, on the basis of analysis of individual teeth, "In both maxilla and mandible, teeth with spacing showed no difference in the amount of periodontal destruction or gingival inflammation from those with contact relationships." They also reported, however, "For the full dentition, as the incidence of spacing increased, periodontal destruction increased."

Others have presented what seem to be contradictory findings. Gould and Picton (11) found that teeth with open or poorly shaped contacts had significantly higher Periodontal Index scores when compared with teeth that had sound proximal contacts. Sanjana and others (12) reported that the percentage of diseased papillae in areas with weak contacts was consistently higher than that found in areas of good contact. Alexander (13) also reported a similar pattern present in a group of two hundred hospital patients. This pattern did not hold true, however, when a similar group of dental students was examined (13).

Thus the therapist is faced with a quandary. Contacts which result in food impaction are a source of annoyance and discomfort to the patient. Clinical impressions and statements of currently acceptable therapy dictate that firm proximal contacts are necessary for gingival health, but reports in the literature reflect conflicting views. Previous studies have not reported the combined influence of plaque and the contact on the periodontal status. Different degrees of plaque removal by the subjects

may account for some of the apparently conflicting views. The purpose of this study was to compare the integrity of the contact with the periodontal status and with the occurrence of calculus, carious lesions, and food impaction.

Methods and Materials

The subjects were 40 young adult male naval recruits, aged 17-19 years, at the Naval Training Center, Great Lakes, Illinois. Each subject was screened for acceptance according to the following criteria:

1. Absence of acute oral disease.
2. Contiguous natural dentition from second molar to second molar in both the maxillary and mandibular arches (14).
3. Absence of known systemic diseases.
4. No dental treatment other than examination and three-agent fluoride treatment since commencing active duty.
5. No temporary proximal restorations.

Each subject was examined using a standard dental unit, chair, light, mouth mirror, and periodontal probe*. Each proximal area was assessed and the findings recorded for the following:

a. Gingival Index (GI) of Loe and Silness (15-16).
b. Pocket depth in millimeters. Pocket depths were recorded when the probe was held parallel to the long axis of the tooth and placed as close to the contact area as possible (17). Both facial and lingual or palatal measurements were made and the greater measurement recorded.
c. Presence or absence of interproximal calculus as determined tactually.
d. Presence of carious lesions recorded with the aid of posterior bitewing radiographs.
e. Presence of proximal restorations.
f. Presence or absence of overhangs noted for each proximal restoration.
g. Food impaction scored as present or absent as determined by the presence of fibrous food wedged interproximally (18).
h. Plaque deposits, determined with the use of a disclosant** were scored as
   (1) none - interdental area free of plaque deposits
   (2) scanty - plaque not extending beyond the line angles
   (3) copious - deposits extending beyond the line angles.
i. Integrity of the contact. Each contact was tested twice with a double strand of unwaxed dental floss*** as described by O'Leary (8). Each contact was described as
   (1) Tight - definite resistance to the passage of floss.
   (2) Loose - minimal resistance.
   (3) Open - no resistance.

*Williams Periodontal Probe, Hu-Friedy, Chicago, IL.
**Trace, Lorvic Corp., St. Louis, MO.
***POH Unwaxed Dental Floss, Oral Health Products, Inc., Tulsa, Oklahoma.

Prior to data collection, the examiners (CVM and EBH) standardized their examination procedures. Inter-examiner variation was determined by performing duplicate examinations on 4 subjects (104 proximal areas assessed). Inter-examiner agreement was better than 80% for measurements of GI, plaque, calculus, and overhangs, and better than 90% for the data on type of contact, food impaction, and presence of restorations. Intra-examiner variation was determined in a similar manner and agreements were better than 90% for all evaluations.

Results

A total of 1040 interdental areas were examined in 40 subjects. Of the 1040 areas, 841 (80%) exhibited signs of moderate or severe gingival inflammation (GI 2 or 3). Figures 1 and 2 illustrate the frequency of the Gingival Index scores in the anterior and posterior regions respectively. Chi-square analysis for the anterior region revealed a significant relationship ($p < .01$) between the GI score and the amount of plaque present. Without exception the subjects had interdental plaque in every area. Only 2% of the anterior areas were considered healthy, while none of the posterior areas were considered to be free of inflammation. The data suggests that when copious plaque deposits were present there was a shift from GI 1 to GI 2. Figure 2 also illustrates that conditions were generally worse in the posterior areas, i.e. approximately 95% of the areas exhibited bleeding on light probing (GI = 2 or 3).

Periodontal pockets 4 mm or greater in depth were present in 29 of the 40 subjects (73%). Figures 3 and 4 illustrate the frequency of pocket scores in millimeters for the anterior and posterior regions. Pockets 4 mm or deeper occurred most commonly in the posterior region. In addition, Table 1 gives the mean pocket depths in millimeters for each region by type of contact present. The type of contact did not appear to have any direct effect on the pocket depth. When copious plaque deposits were present, though, the pocket depths tended to be greater. In addition to an increased bleeding tendency, loss of attachment was more common with copious deposits of plaque.

Forty-two areas, or 4% of all interdental areas, exhibited food impaction. Table 2 summarizes the findings for the mean GI and pocket depths by type of contact for these areas. There was little change in the GI score when the type of contact was evaluated. The pocket depth was least for those areas with tight contacts, intermediate for those with loose and greatest for those with open contacts.

Chi-square analysis revealed a significant relationship ($p < .01$) between the distribution of types of contact and occurrence of food impaction (Table 3). Food impaction occurred most frequently when loose contacts were present and least often when the contacts were tight. The effects of food impaction on pocket depth are also presented in Table 3 where the mean pocket depths for interdental areas with food impaction are compared with the mean pocket depths for areas without food impaction. For each type of contact, it is seen that the mean pocket

depth where food impaction was present was greater than the mean pocket depth found for that type contact. The differences between groups were not significant for the tight contact areas, but were significant ($p < .01$) for the loose and open contact areas.

Calculus deposits were detected in 170 (16%) of the interproximal areas. The relationship between calculus and contact type, with or without food impaction, was not significant. Figure 5 illustrates the frequency of occurrence of calculus at each pocket depth. This relationship was significant ($p < .01$). Thus, as pocket depths increased, the pockets were more likely to have calculus deposits.

A total of 30 proximal carious lesions were detected in 10 of the 40 subjects. A meaningful analysis of the effect of proximal carious lesions on the periodontal status was not possible because of the small numbers involved.

Seventy-six proximal restorations were present. These were clustered in 14 subjects. Either calculus or overhangs were associated with 36 (46%) of these restorations. There were too few proximal restorations to conduct an analysis of the effect on contact type and food impaction.

Discussion

The study population was a highly select group of young, adult males who were undergoing the rigors of recruit training. In addition, the criteria for selection were such that these subjects could be considered as those with the highest level of oral health among naval recruits. In spite of this, they all had interdental plaque and more than 80% of the areas examined were scored as exhibiting moderate or severe gingival inflammation, i.e. bleeding on light probing. The occurrence of pockets 4 mm or greater in depth in 73% of the population confirms work previously reported in naval recruits using Navy Periodontal Screening Exam partial mouth recordings (19).

The findings of this study indicated considerably fewer open and loose contacts than previously reported (8). This difference may be attributed to several factors such as age differences in the subjects, different levels of dental care, full and partial mouth recordings, and the fact that both populations were highly select groups not necessarily indicative of the general population.

The 4% occurrence of food impaction noted in this study may be an underestimation of the problem, as only fibrous material wedged interproximally was considered as food impaction. Soft deposits, or deposits which impacted upon the gingiva but were not retained, were not scored.

Food impaction was most commonly associated with loose proximal contacts. Other factors are also thought to be involved, such as location and area of the contact, marginal ridge integrity, and plunger cusp mechanisms. These and other suspected mechanisms bear further investigation.

The finding that 46% of the proximal restorations were involved with calculus or overhangs was quite discouraging especially when the occurrence of calculus was 16% for the entire population. Areas of mechanical retention for bacterial plaque are almost impossible for the patient to maintain. The clinician must be meticulous in his finishing of the gingival margins of restorations to prevent such occurrences. These findings indicate that more attention is needed in this area.

Summary and Conclusions

A group of 40 healthy, young adult male naval recruits were examined and scored for gingival inflammation, plaque, food impaction, pocket depth, carious lesions, calculus, restorations and overhangs. The findings of this study support those previously reported in naval recruits, i.e. gingival inflammation is widespread involving almost every area examined. Naval recruits do not adequately remove deposits of interdental plaque, and at least one pocket with a depth of 4 mm or greater is present in 3 out of every 4 recruits. Additionally, it was found that the level of gingival inflammation was considered moderate or severe in more than 80% of the areas examined.

Analysis revealed no significant relationship between contact type and GI or pocket depth. However, the significant relationship observed between food impaction and contact type, and between food impaction and pocket depth, reinforced clinical observations that food impaction contributes to periodontal pathology. A discouragingly high number of restorations had mechanical retentive areas for bacterial plaque accumulations. Thus, in addition to establishing adequate levels of interproximal surface plaque removal by the patient, the clinician should take great care in finishing the gingival margins of restorations and should eliminate those factors associated with food impaction early in treatment.

References

1. Waerhaug, J. Effect of rough surfaces upon gingival tissue. J. Dent. Res. 35:323, 1956.

2. Glickman, I. Clinical Periodontology, 4th ed., Philadelphia, W. B. Saunders Co., 1972, p. 492.

3. Goldman, H. M. and Cohen, D. W. Periodontal Therapy, 5th ed., St. Louis, C. V. Mosby Co., 1973, p. 1029.

4. Grant, D. A., Stern, I. B., and Everett, F. G. Orbans Periodontics, 4th ed., St. Louis, C. V. Mosby Co., 1972, p. 673.

5. Prichard, J. Advanced Periodontal Disease: Surgical and Prosthetic Management, 2nd ed., Philadelphia, W. B. Saunders Co., 1972, p. 17.

6. Ramfjord, S. Local factors in periodontal disease. J. Am. Dent. Assoc. 44:647, 1952.

7. Stahl, S. S. The etiology of periodontal disease - review of the literature. In Ramfjord, S. P., Kerr, D. A. and Ash, M. M. eds.: World Workshop in Periodontics, Ann Arbor, University of Michigan, 1966, pp. 127-145.

8. O'Leary, T., Baudell, M. C., and Bloomer, R. S. Interproximal contact and marginal ridge relationships in periodontally healthy young males classified as to orthodontic status. J. Periodontol. 46:6, 1975.

9. Larato, D. C. Relationship of food impaction to interproximal intrabony lesions. J. Periodontol. 42:237, 1971.

10. Geiger, A. M., Wasserman, B. H., and Turgeon, L. R. Relationship of occlusion and periodontal disease. Part VIII - Relationship of crowding and spacing to periodontal destruction and gingival inflammation. J. Periodontol. 45:43-9, 1974.

11. Gould, M. S. E. and Picton, D. C. A. The relation between irregularities of the teeth and periodontal disease. Brit. Dent. J. 121:20, 1966.

12. Sanjana, M. K., Mehta, F. S., Doctor, R. H., and Shroff, B. C. Incidence of localized periodontal disease of the interdental papilla in the premolar molar region and its relation to the dimensions of the contact points concerned. J. All India Dent. Assoc. 28:201, 1956.

13. Alexander, A. G. Periodontal aspects of conservative dentistry. Brit. Dent. J. 125:111, 1968.

14. Beagrie, G. S. and James, G. A. The association of posterior tooth irregularity and periodontal disease. Brit. Dent. J. 123 239, 1962.

15. Loe, H. and Silness, J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odont. Scand. 21:533, 1963.

16. Loe, H. The gingival index, the plaque index and the retention index systems. J. Periodontol. 38:610, 1967.

17. Tibbets, L. S. Use of diagnostic probes for detection of periodontal disease. J. Am. Dent. Assoc. 78:549, 1969.

18. Hirschfield, I. Food Impaction. J. Am. Dent. Assoc. 17:504, 1930.

19. United States Navy, Naval Dental Research Institute. Summaries of Research Fiscal Year 1977. Naval Medical Research and Development Command, Bethesda, MD NDRI-PR 77-07.

Table 1. Mean Pocket Depth in Millimeters for Each Type of Contact

	Type of Contact		
Hygiene Status	Tight N=378	Loose N=87	Open N=95
Anterior Region			
Scanty Plaque	2.63 mm	2.62 mm	2.59 mm
Copious Plaque	2.71 mm	2.70 mm	2.65 mm
Posterior Region			
Scanty Plaque	2.94 mm	3.00 mm	3.00 mm
Copious Plaque	3.12 mm	2.98 mm	3.39 mm

Table 2. Mean GI Score and Pocket Depths in Millimeters for Teeth Exhibiting Food Impaction

	Type of Contact			
Periodontal Status	Tight N=15	Loose N=21	Open N=6	Total N=42
Anterior				
GI	2.00	1.79	2.00	1.99
Pocket Depth	2.85	3.00	3.75	3.06
N =	10	12	4	26
Posterior				
GI	2.00	2.00	2.00	2.00
Pocket Depth	3.00	3.00	3.50	3.06
N =	5	9	2	16
Total Mean Pocket Depth	2.90	3.00	3.67	3.06

Table 3. Effect of Food Impaction on Pocket Depth in Millimeters in Various Types of Interdental Contacts

	Without Food Impaction	With Food Impaction
Tight	2.89 (N=804)	2.90 (N=15)
Loose	2.68 (N=94)	3.00 (N=21)
Open	2.64 (N=100)	3.67 (N=6)
Population Mean	2.86 (N=998)	3.06 (N=42)

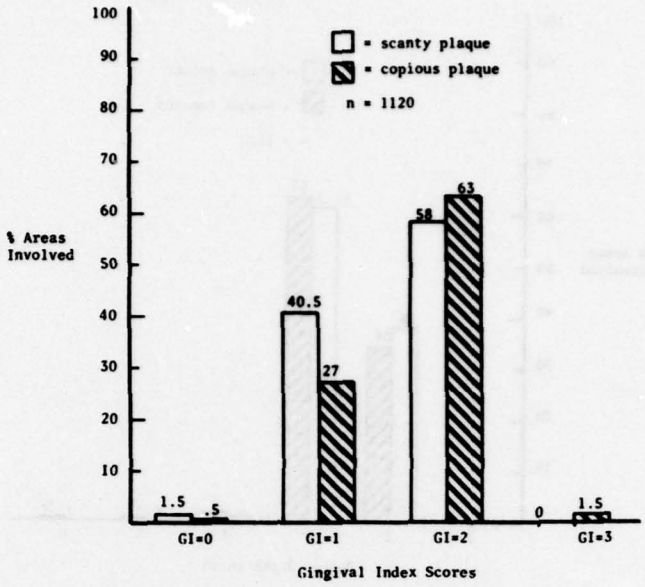

Figure 1. Frequency of Gingival Index scores in the anterior region compared with plaque deposits.

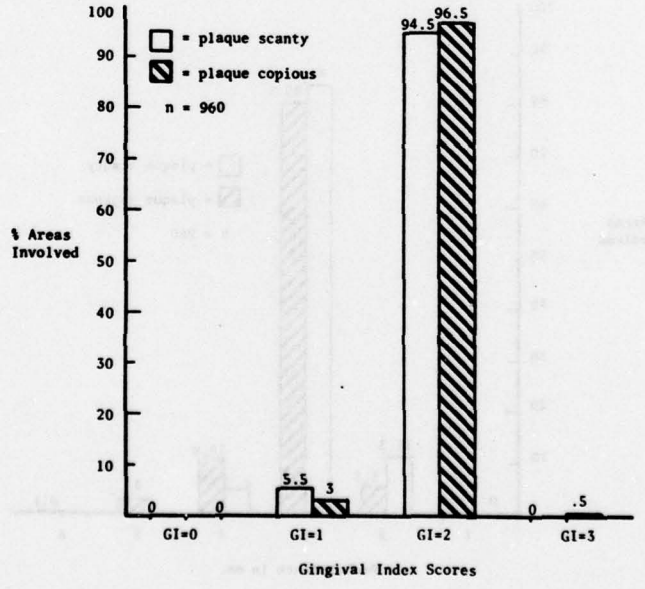

Figure 2. Frequency of Gingival Index scores in the posterior region compared with plaque deposits.

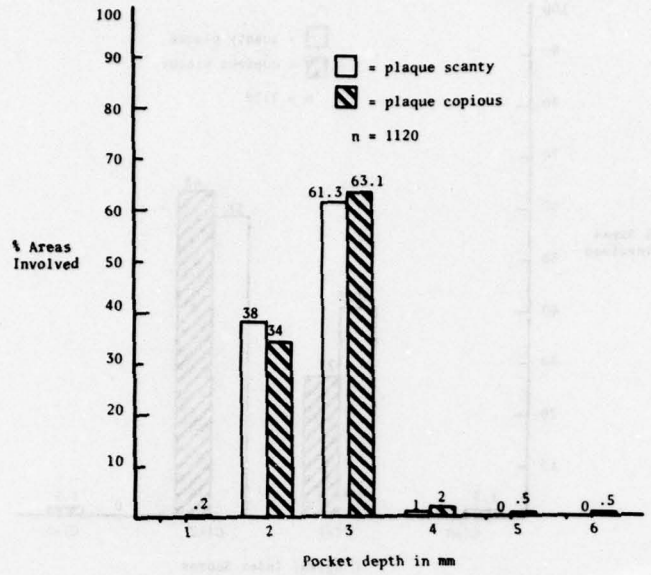

Figure 3. Frequency of pocket depths in millimeters in the anterior region compared with plaque deposits.

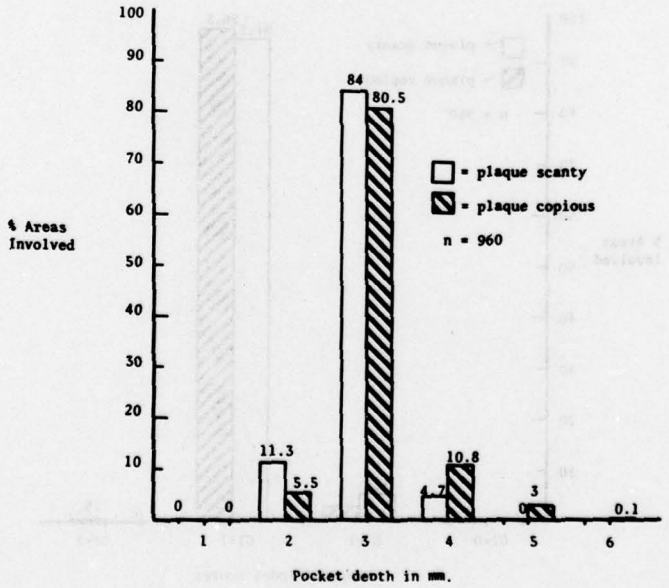

Figure 4. Frequency of pocket depths in millimeters in the posterior region compared with plaque deposits.

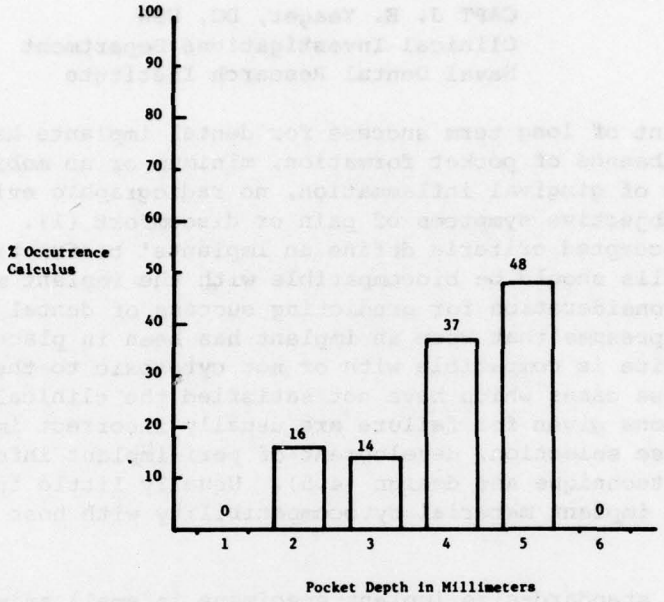

Figure 5. Percent occurrence of calculus at a given pocket depth.

CYTOTOXICITY OF FIVE DENTAL IMPLANT MATERIALS

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The establishment of long term success for dental implants has utilized several criteria: absence of pocket formation, minimum or no mobility of the implant, absence of gingival inflammation, no radiographic evidence of bone loss, and no subjective symptoms of pain or discomfort (1). The fulfillment of clinically accepted criteria define an implants' biofunctionality. However, the host cells should be biocompatible with the implant material as another primary consideration for predicting success of dental implants (2,3). Most investigations presume that when an implant has been in place successfully for 5 years, the device is compatible with or not cytotoxic to the surrounding host cells. For those cases which have not satisfied the clinical criteria for five years, the reasons given for failure are usually incorrect implant design, poor bone site or case selection, development of peri-implant infection, and improper prosthetic technique and design (4,5). Usually little information is available concerning implant material cytocompatibility with host peri-implant tissues.

Implantation of standard-size implant specimens in small animals followed by microscopic evaluation of the membrane surrounding the implant is a technique widely used for the evaluation of biocompatibility of implant materials. Thickness and histopathlogic condition of the membrane are assumed to reflect the cytotoxicity of the reaction between the host tissue and implant materials. It has become increasingly apparent this is an unsatisfactory technique since the thickness and cellularity of the fibrous membrane may depend on mechanical interaction of the implant with adjacent tissue as well as on cytotoxic effects (3).

The purpose of this investigation was to determine the cytotoxicity of five dental implant materials: vitreous carbon, aluminum oxide, acrylic, titanium, and ticonium. If these materials did not demonstrate cytotoxic effects they might be likely candidates for dental implant materials.

Vitreous carbon is a hard, impermeable, crystalline structure (6,7). It is formed by the carbonization of thermosetting resins (8). It contains less than 200 ppm impurities and is chemically inert (7). Studies have shown vitreous carbon to have almost total biocompatibility with human tissues (6,9). Its physical properties are very suitable for use in implants, being strong yet easily molded and machined (7).

Aluminum oxide (Al_2O_3) or alumina has a crystalline structure which makes it highly porous. The alumina implants are available in three densities which are labeled porous ($3.24 \pm .05$ g/CM^3), semi-dense ($3.50 \pm .05$ g/CM^3) and dense ($3.95 \pm .05$ g/CM^3) (10). Commercially pure alumina (99.4%) contains a large number

of impurities which include Si, Ca, Mg, Cr, Fe, Ti, Ma, Na, K, and Li (9). These impurities are thought to cause a negative tissue response and the resultant fibrotic encapsulation of alumina implants. A recent investigation noted alumina elicited more tissue response than vitreous carbon (9). The difference in compatibility has been blamed on impurities and surface roughness of alumina implants, as well as lack of electric conductivity of alumina as compared to vitreous carbon.

Acrylic is highly desireable as an implant because of its strength, and ease of fabrication. It is formed by a polymerization reaction between a monomer (methylmethacrylate) and its polymer (polymethylmethacrylate). The tissue response to acrylic is questionable. Stinson (11) indicated a favorable tissue response to acrylic while others (12) have indicated acrylics may be carcinogenic. Unfavorable tissue response, when it did occur, seemed to stem from the presence of an excess free monomer due to incomplete polymerization (13).

Titanium is a strong and highly malleable metal. Commercially pure titanium contains 0.25% iron, 0.03% carbon, 0.64% nitrogen and 99.08% titanium. Its high degree of purity gives it an extremely high level of resistance to corrosion and makes it more compatible with tissues than an alloy. Studies have indicated titanium was compatible with viable tissues (14, 15). The capsules which formed around titanium implants were consistently thin, indicating minimal tissue reaction. Due to its biocompatability and ready availability, titanium has been used in approximately 90% of all dental metallic implants (16).

Ticonium is a highly ductile and acid resistant alloy. It is composed of 54.3% Ni, 24.6% Cr, 15.4% CO, 4.4% Mo, 0.57% Si, 0.6% Fe, and a negligible amount of carbon (17). Ticonium would be a reactive material in tissue fluids if it were not for an inert oxidized film which forms over the metal's surface (3).

MATERIALS AND METHODS

Vitreous carbon,* porous grade aluminum,** self curing acrylic,*** blade-vent titanium,**** and ticonium***** dental implant materials were shaped into 1 x 3 mm round disks. Paper disks of similar dimensions were utilized as controls. Implant disks were cleaned ultrasonically in alkaline cleaner, rinsed in deionized water, and air dried. Titanium and ticonium disks were also passivated according to the recommended procedure of American Society for Testing amd Materials (19). Passivation included placement of the two metal implant disks in 40% nitric acid at 50°C for 1/2 hour. Nitric acid treatment was followed by a deionized water rinse and air drying. Dried implant and control disks were sterilized in ethylene oxide and aired 48 hours in their sterilizing packs prior to exposure to cultured cells.

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***Acralite 88, Kerr Products, Romulis, Michigan.
****Implant Research Corporation, Box 123, Pennasauxen, N. J.
*****Ticonium, 413 N. Pearl St., Albany, N. Y.

Cell Culture Procedures

Gingival fibroblast cells were used in the experimental procedure. Cells originated from a primary culture (GF1) of a gingival specimen obtained from the retromolar area of the right mandibular second mola of a 32 year old Caucasian male. The excised specimen was rinsed thoroughly in deionized water, stripped of epithelial tissue and cut into 25-30 pieces which were placed on the bottom of a petri dish,* 60 x 15 mm. Five ml of primary culture medium (PCM) was slowly added to the dish. The PCM consisted of 85% Dulbecco-modified Eagles medium** and 15% fetal bovine serum.*** The PCM was supplemented with 0.225 gm% bicarbonate and 500 I.U. benzyl penicillin, 400 ug streptomycin sulfate, and 100 ug kanamycin sulfate antibiotics per ml.

When the fibroblast cell monolayer reached confluency, the primary culture was harvested by trypsinizing the cells and subculturing them in cell culture medium (CCM) (20). Trypsinizing medium was the Dulbecco medium described above, supplemented with 0.25% trypsin*** and 0.225 gm% bicarbonate. Cell culture medium consisted of 90% Dulbecco medium and 10% fetal bovine serum, with 100 I.U. benzyl penicillin and 100 ug kanamycin sulfate added per ml. Viable fibroblast cells were maintained by subculturing a parent culture into two subcultures when the parent monolayer reached confluency. All cultures were incubated at 37°C in a humidified environment with 5% CO_2.

Dental implant materials were tested in cytotoxicity testing medium (CTM) which consisted of CCM and 1.5% methylcellulose (18). At the end of the cytotoxicity testing period, the fibroblast monolayers were stained for viable cells by cytotoxicity staining medium (CSM) which contained CCM supplemented with agar to a final concentration of 1.5% and 0.006% neutral red stain. Neutral red is a non-toxic vital stain. It stains viable cells in preference to non-viable cells. Clear areas indicate areas of non-vital cells while areas of diffuse red staining indicate areas of vital cells.

Another gingival specimen was obtained from the right maxillary tuberosity area of a 34 year old Caucasian male and was prepared as described above to result in a second primary culture of gingival fibroblasts (GF2). Growth and maintenance of cells from GF2 were the same as for GF1. Cells from GF2 were used in a second cytotoxicity testing period.

Experimental Procedure

On the first experimental day, trypsinized fibroblasts from a confluent GF1 culture were counted in a hemacytometer and 400,000 cells subcultured in each of four petri dishes, 60 x 15 mm, containing 5 ml CCM. On the fourth day each experimental culture was washed two times with 3 ml phosphate-buffered saline, pH 7.4, and then gently overlayed with 3 ml of CTM which had been chilled to 4°C. The four experimental cultures were then warmed to 37°C in the incubator for one hour. At this temperature the CTM becomes a viscous gel. The implant disks (round 1 x 3 mm disks), vitreous carbon, aluminum oxide, acrylic, titanium, and ticonium were placed on the upper

*Lux Scientific Corp., Newbury Park, California.
**Biolabs, Inc., Northbrook, Illinois.
***Rehiss, Kankakee, Illinois.

surface of the gelled-CTM and gently eased down through the medium until they came to rest immediately over the growing fibroblast monolayer.

Vitreous carbon, aluminum oxide, and ticonium were placed into each of two cultures and acrylic and titanium into each of the two remaining cultures. One positive and negative control paper disk were also placed into all four cultures. The positive controls had 0.05 ml of 5% phenol and the negative controls 0.05 ml of CCM absorbed into them just prior to placement. The disks were placed an equal distance from each other in all four cultures. The gel-like CTM maintained all disks in a stationary position and apparent contact with the fibroblast monolayers throughout the cytotoxicity testing period as long as the cultures were handled gently and maintained in a level position.

On the sixth experimental day, 3 ml of CSM was gently layered over each of the four cultures. On the seventh day, the fibroblast monolayer of each culture was inspected for cellular staining by neutral red, a constituent of the CSM.

The monolayer area surrounding each disk, implant and control, was labeled N or P according to the following criteria:

N = No apparent reaction by the cells to the disk, cell integrity and monolayer appeared unaffected, cells take up neutral red stain and monolayer area appeared red in color.

P = An apparent reaction by the cells to the disk, cells appear necrotic with loss of cellular integrity and disruption of monolayer, cells do not take up neutral red stain and monolayer area appeared clear or very light pink red due to presence of stain in overlying CSM.

The five implant materials were subjected to a second cytotoxicity testing period using GF2 fibroblast cells. Experimental procedures were as described above for GF1 cells.

RESULTS

The influence of vitreous carbon, aluminum oxide, acrylic, ticonium, and titanium on the cytology of gingival fibroblasts, GF1 and GF2, as indicated by neutral red staining and morphological appearance are summarized in Table 1. Cells surrounding the medium-impregnated control disks and all the implant disks appeared to be vital. The cells maintained their integrity and characteristic fibroblastic shape. Neutral red appeared to have stained all the cells.

The cells surrounding the phenol-impregnated control disks appeared to have complete loss of cell architecture and integrity. None of the fibroblasts surrounding the phenol disks had taken up neutral red stain. They appeared to be non-vital cells.

DISCUSSION

Layering the fibroblast monolayer with cell culture medium, supplemented with methyl cellulose, allows placement of solid implant materials in close approximation with viable cells. The viscosity of the methyl cellulose-cell culture medium permited gentle positioning of the solid materials immediately above the monolayer and yet maintained the solid materials in a stationary position without mechanically disrupting the cell monolayer. Maintaining solid materials in apparent contact with the monolayer during the cytotoxicty testing period would seem to be an advantage over suspending the test materials about 2-3 mm above the cells as described for the cell culture medium-agar overlay technique (21).

No final, definite conclusions can be drawn from a cytotoxicity test period of 48 hours. The test does not reveal all possible host responses to the solid implant materials such as immunologic and chronic cytotoxic effects. Valid observations of a preliminary nature can be made and the cytotoxicity test may predict which implant materials would be biologically acceptable to host tissue. Materials which cause disruption and death in contact with a fibroblast cell monolayer would not be promising candidates for biologically acceptable dental implants.

At the end of the staining period it was observed that the fibroblasts around the medium-impregnated control disk (negative control) and around each implant disk took up the neutral red stain. Microscopic examination revealed the stain concentrating in the cell nuclei and that there appeared to be no disruption of the cell monolayer or alteration of cell morphology. On the other hand fibroblasts around the phenol control disks (positive control) were totally destroyed with only cell outlines visible upon microscopic examination. These results suggest that the implant disks had no affect on the vitality of the fibroblasts and that vitreous carbon, aluminum oxide, acrylic, titanium, and ticonium would be cytologically-compatable implant materials.

References

1. Babbush, C. A. and Greene, A. H. Implant dentistry: A long term survey and comparative study with fixed bridge work. Oral Implantology 7:89-105, 1977.

2. Kent, J. N., De Long, P., Homsy, C. A. and Hinds, E. C.: Intraosseous dental implants: Nature of the interface. J. Amer. Dent. Assoc. 89: 1142-1151, 1974.

3. Homsy, C. A., Kent, J. N. and Hinds, E. C. Materials for oral implantation - biological and functional criteria. J. Amer. Dent. Assoc. 86:817-832, 1973.

4. Viscido, A. J. Prosthodontics for the submerged functional implants. Oral Implantology 6:193-201, 1975.

5. Keene, D. M. Periodontal considerations for implant dentistry. The Dent. Clin. N. Amer. 20:155-179, 1976.

6. Grenoble, D. E. and Kim, R. Progress in the evaluation of a vitreous carbon endosteal implant. Ariz. State Dent. J. 19:12-19, 1973.

7. Benson, J. Elemental carbon as a biomaterial. J. Biomed. Mater. Res. Symp. No. 2, Part 1, 41-47, 1971.

8. Kaae, J. L. The mechanical properties of glossy and isotrophil pyrolytic carbons. J. Biomed. Mater. Res. 6:279-282, 1972.

9. Hobkirk, J. A. Tissue reactions to implanted vitreous carbon and high purity sintered alumina. J. Oral Rehabilitation 4:355-368, 1977.

10. Witlock, R. P. Personal communication, U. S. Dept. of Commerce, National Bureau of Standards, Gaithesburg, MD.

11. Stinson, N. E. The tissue reaction induced in rats and guinea pigs by polymethylmethacrylate (acrylic) and stainless steel. Brit. J. Exp. Path. 45:21-29, 1963.

12. Shklar, G., Hodosh, M. and Povar, M. Tissue reactions to the plastic tooth implant. J. Oral Surg., Oral Med., Oral Path. 22:349-357, 1966.

13. Brown, D. E. Tissue reaction to plastic and metal implants. Arch. Otolaryng. 88:283-287, 1968.

14. Hirschon, J. S., McBeath, A. A. and Dustoor, M. R. Porous titanium surgical implant materials. J. Biomed. Mater. Res. Symp. No. 2, Part 1, 49-67, 1971.

15. Zaller, R. D. and Fitzgerald, B. E. Treatment of mandibular fractures with use of malleable titanium mesh: report of 56 cases. J. Oral Surg. 34:748-754, 1976.

16. Weiss, C. M., Judy, K. W. M. and Chiarenza, A. R. Precompacted, coined titanium endosteal blade implants. Oral Implantology 3:237-260, 1973.

17. Civjan, S. Properties of surgical casting alloys. J. Prosth. Dent. 28:71-81, 1972.

18. Casto, B. Personal communication, Biolabs, Inc., Northbrook, IL.

19. Standard recommended practice for surface requirements of metallic surgical implants. In: Annual Book of ASTM Standards, pp. 296-297, American Society for Testing and Materials, Easton, MD, 1975.

20. Litwin, J. Trypsinization of diploid human fibroblasts. In: Tissue Culture, Kruse, B. F. and Patterson, M. K., Jr., eds., pp. 188-192, Academic Press, 1973.

21. Powell, D., Lawrence, W. H., Turner, J., and Autian, J. Development of a toxicity evaluation program for dental materials and products. 1. Screening for irritant responses. J. Biomed. Mater. Res. 4:583-596, 1970.

TABLE I

Cytotoxicity of Five Dental Implant Materials in 48 Hour Gingival Fibroblast Culture

Culture	Implant Material	Control	Cytological Effect GF1*	GF2*
1	Vitreous Carbon		N	N
	Aluminum Oxide		N	N
	Ticonium		N	N
		5% phenol	P	P
		CCM	N	N
2	Acrylic		N	N
	Titanium		N	N
		5% phenol	P	P
		CCM	N	N
3	Vitreous Carbon		N	N
	Aluminum Oxide		N	N
	Ticonium		N	N
		5% phenol	P	P
		CCM	N	N
4	Acrylic		N	N
	Titanium		N	N
		5% phenol	P	P
		CCM	N	N

N = No apparent adverse cytological reaction by fibroblast monolayer.
P = An apparent adverse cytological reaction by fibroblast monolayer.
*Cells from GF1 and GF2 primary cultures.

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This report contains the final reports of the research conducted by the Dental Students assigned to the Naval Dental Research Institute during the summer of 1978. The project titles are as follows:

FLUORIDE ACCUMULATION BY DENTAL PLAQUE MICROORGANISMS,
INFLUENCE OF INTERDENTAL CONTACTS ON PERIODONTAL STATUS and
CYTOTOXICITY OF FIVE DENTAL IMPLANT MATERIALS.

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