Original Article

Cytotoxic effect of indigenously fabricated dental magnets for application in prosthodontics

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Abstract

Context: Dental magnets are used for retaining removable prostheses such as a removable partial denture, complete denture, and maxillofacial prosthesis. They provide good retention for the prostheses. However, the elements released from the magnets may be cytotoxic for the tissues. Therefore, it is necessary to evaluate their cytotoxic effect on cell lines.

Aim: The aim of the study is to check the cytotoxic effect of indigenously fabricated dental magnets on animal cell lines.

Materials and Methods: Neodymium-iron-boron (Nd-Fe-B) magnet was tested for cytotoxicity. The magnet was encased in a teflon cylinder. Magnets were placed in the well tissue-cultured plates together with a suspension containing NIH 3T3 mouse fibroblasts (5×10^5 cells/ml). After 3 days of incubation at 37°C, cell viability was determined by mean transit time (MTT) assay. Cells were subsequently dissolved in 100 µl dimethyl sulfoxide with gentle shaking for 2 h at room temperature followed by measurement of absorbance at 570 nm. Eight replicate wells were used at each point in each of four separate measurements. Measured absorbance values were directly used for calculating percent of viable cells remaining after the respective treatment. Data were analyzed statistically with significance level set at $P < 0.05$.

Results: The control group had highest absorbance reading for the MTT assay followed by test group. The lowest values were found with bare Nd-Fe-B magnets. One-way ANOVA test was performed for the data obtained. There was a statistical significant difference seen in the positive control (bare magnets, 44.96) and the test (teflon cased magnets, 96.90) group.

Conclusion: More number of viable cells was visible in test group cells indicating that the indigenously fabricated dental magnet did not show any cytotoxicity.

Keywords: Cytotoxicity, dental magnet, neodymium-iron-boron magnet

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INTRODUCTION

Metal alloys used in dentistry for various applications that place them into contact with the oral epithelium, connective tissue, or bone for many years. Since these metals are in contact with the tissues for long-term, it is of paramount that the biocompatibility of casting alloys be measured

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and understood. All biomaterials used in dentistry must be evaluated for biocompatibility. This has generated a requirement for cytotoxic assays to screen materials that may cause potentially harmful effects of a material to oral tissues before clinical use.

Biocompatibility may be defined as the ability of a material to induce an appropriate and advantageous host response during its intended clinical use. The traditional concept of biocompatibility is regarded as a lack of significant adverse reaction between the oral tissues.[1] Dental material biocompatibility has long been described throughout the dental literature; however, information about the factors that determine biocompatibility responses is only just emerging. The term cytotoxicity is used to describe the cascade of molecular events that interfere with macromolecular synthesis, causing unequivocal cellular and functional and structural damage.[2]

Prosthetic constructions retained by magnets play an increasing role in the application of dental implants, for dental combination prosthesis, and orofacial epithesis.[3-6] Rare earth magnetic alloys such as samarium-cobalt (Sm-Co), neodymium-iron-boron (Nd-Fe-B), and others have a considerable high magnetic strength.[7] Therefore, the magnetic force necessary for dental and other applications can be obtained with very small magnets. Nd-Fe-B magnets have replaced the Sm-Co due to their maximum energy, which is in the range of 36–50 Mega Gauss Oersted (MGOe). They are easily available in the form of big rods, which can be machined cut to desired dimension required for the dental purpose. Therefore, the Nd-Fe-B magnet was chosen for the study.[7]

Although a new magnetic material samarium-iron nitride has been suggested in the literature, a potential replacement to be developed as a dental magnet.[8] However, studies done on this material till date are not able to generate the maximum energy. They are only capable of producing maximum energy of 10.5 MGOe.[9]

One of the issues during the introduction of these magnetic materials into clinical practice was if the high magnetic field strength applied might exert some negative impact on the surrounding tissue. Although the dental magnets are encased in a corrosion-resistant material, there may be a chance that the sealed gap between the lid and the body may open up and start corroding the magnet.[9] The corrosion products released from the magnet may be cytotoxic and may cause damage to the cells.[7] Materials used in various dental applications are exposed to mechanical loading and pH causing rapid corrosion of metallic materials.[10] Metal ions are released from dental materials *in vitro* and *in vivo*.[11,12] Released metal ions in dental and other applications can cause staining of the surrounding tissue, mild to severe local inflammation up to systemic effects, such as sensitivity and allergic reactions.[13,14] Negative effects that were observed in the surrounding tissue were consequently attributed to a positive toxic effect of magnetic corrosion products and not to the static magnetic fields.[15]

In the present study, indigenously fabricated dental magnet was encased with teflon sleeve and was tested for cytotoxicity to see whether there will be release of any corrosion products. The aim of the study was to check the cytotoxic effect of indigenously fabricated dental magnet. The magnet is made of Nd-Fe-B and encased with teflon. Basically, the magnets have cytotoxic potential in oral environment due to their corrosive nature. Corrosion of dental materials and other implantable materials such as metals poses a serious problem for their usage in clinical scenarios.

MATERIALS AND METHODS

Fabrication of magnet

A rare earth Nd-Fe-B-based magnet was procured from the Magnatech Co. Ltd, Mumbai, India. These are readily available in the form of blocks, rods, and discs. The block type magnet was cut to a desired dimension on a computer numerical control (CNC) micro lathe at Magnatech Engineering Pvt. Ltd., Mumbai, Maharashtra, India. The diameter of the magnet was designed based on the average value of cross-sectional diameter of the mandibular canine and premolar tooth. The diameter of the magnet was set at 3 mm with 1.5 mm thickness. As the bare magnet is readily corroded in the oral environment, it was encased in a corrosion-resistant material. Teflon was used to fabricate a sleeve for embedding the magnet. Teflon is a known biocompatible material and resists corrosion [Figure 1].The sleeve was designed and fabricated at National aeronautical Laboratory, Bengaluru, Karnataka, India. The teflon sleeve was fabricated using CNC micro lathe. The thickness of the sleeve was 0.7 mm.

The specimens were divided into three groups, namely, control, positive control, and the test. Control group was the one with cells without any magnets. Positive control group is nonencased bare magnet, and the test group was teflon sleeve encased magnet.

Cytotoxicity testing

Cell culture

NIH 3T3 mouse fibroblasts were used as an established cell lines for testing the cytotoxicity of dental magnet.

Cell lines were procured from National Centre for cell Sciences; Pune, MS, India. Cells were cultivated in the tissue culture flasks with Dalbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, Mo, USA) containing 10% fetal bovine serum (FBS; Sigma). Once the cells became 80% confluent cultures, they were fed with 2% FBS media. Cells were seeded to 12 (3.9 cm^2) well tissue culture plates at 5000 cells/well.

The specimens were cleaned with 70% ethanol first with gauze pieces and then sterilized in ethylene oxide at 37°C for 4 h. Cytotoxicity of magnets measured by culture of cells in direct contact. The respective magnets test and positive control were immersed in the culture medium for 72 h at 37°C. The control group had just the cells without any treatment. Positive control had the bare nonencased dental magnet, and the test group had the magnet with teflon sleeve.

After 72 h time point, the cell morphology was observed. Further mean transit time (MTT) assay was performed based on standard methodology.[16] The media was aspirated, cells were washed with FBS, and 200 μl of 5 mg/ml MTT was introduced to each well and incubated for 2 h.

The plates were centrifuged and the medium was decanted. Cells were subsequently dissolved in 600 μl of dimethyl sulfoxide with gentle shaking for 2 h at room temperature. It was followed by measurement of absorbance at 570 nm. 8 replicate wells were used at each point in each of four separate measurements. The total sample size was 30, and from the 4 measurements, 32 readings were recorded, out of which 30 were taken into consideration. Measured absorbance values were directly used for calculating percentage of viable cells remaining after the respective treatment. In a simultaneous experiment, cells were washed in FBS and performed with DNA fragmentation assay to assess the DNA damage.

Figure 1: Indigenously fabricated dental magnet

The percentage of viability values was calculated as the optical density reading of the probe divided by the optical density reading of the control multiplied by 100. The data obtained were subjected to statistical analysis with a significant set at $P < 0.05$.

RESULTS

The control group had highest absorbance reading for the MTT assay followed by the test group. The lowest values were found with bare Nd-Fe-B magnets. The optical density measurements have been displayed in Table 1. One-way ANOVA was done to test whether there was a difference in means of three groups (between control, positive control, and test). Test showed a significant difference in the mean quantitative cell viability percentages between positive control and test groups. On further Tukey's *post hoc* analysis, it was found that the mean quantitative cell viability percentage of the test group was greater than positive control group [Table 2]. The control had displayed complete cell viability, as there was no magnet introduced in it.

Table 1: Quantitative cell viability: Percentage values of optical densitometry at 570 (nm) after values of the mean transit time test for control, positive control, and test specimens

Specimens	Control	Positive control	Test
1	100	42	95
2	99	39	98
3	100	54	97
4	101	38	96
5	100	42	94
6	98	39	98
7	100	54	96
8	100	38	95
9	100	41	94
10	100	48	98
11	99	51	97
12	100	52	98
13	100	50	98
14	99	42	98
15	98	54	100
16	100	40	95
17	100	42	95
18	99	38	99
19	100	52	100
20	100	38	97
21	100	46	97
22	100	42	98
23	100	53	96
24	99	38	95
25	100	42	94
26	100	38	100
27	100	52	96
28	100	53	95
29	98	53	98
30	99	38	100

The MTT test data [Figure 2] revealed that control cells without any treatment were 99.6% viable compared to positive control, where the samples treated were bare magnet, which is cytotoxic, and only 44.96% viable cells were found after the treatment. On the other hand, our test sample, which was teflon sleeve encased magnet, could protect the cells from the concealed magnet and showed the viability of 96.9%.

To further support our MTT data, cell morphological findings represented no toxic reactions in the control group, where full-grown healthy cells were visible [Figure 3]. The picture was taken with an inverted microscope (Moticam 5; Motic Asia, Kowloon, Hongkong). Positive control slide showed necrotic round cells [Figure 4]. The test cells were healthy with good proliferation demonstrating teflon casing prevents cytotoxic nature of concealed magnet [Figure 5].

Further DNA damage study was conducted to analyze the magnet-induced toxicity in NIH 3T3 cells. DNA fragmentation was noticed in the positive control group, compared to no DNA damage seen in control and test groups [Figure 6], where full-length DNA band was visible near the origin.

DISCUSSION

The indigenously fabricated dental magnet was encased in a teflon cylinder. The teflon cylinder housed the Nd-Fe-B magnet, and it was sealed with a teflon lid using cyanoacrylate resin. Bondemark *et al*.[17] conducted cytotoxic study on Sm-Co and Nd-Fe-B magnets. They showed that the polymeric-based material coating on these magnets such as parylene and teflon had negligible cytotoxicity. Short-term exposure to a static magnetic field did not cause any cytotoxic effect on the cells.

In the present study, direct contact *in vitro* cytotoxic test was conducted. Direct contact between the test specimen and cells as a further possibility combines possible toxic material effects with the influence of the physiochemical nature of the stratum on the cell directly.[18] In the cytotoxic evaluation done by MTT assay, living cells and percentage of cell viability was 99.9% in the control group, 97% in the test group, and 46% in the positive control group. After 72 h culture of fibroblasts, the positive control group had stronger toxicity because cells in the vicinity were rounded

Table 2: Comparison of mean quantitative cell viability percentage values of optical densitometry after values of the mean transit time test for control, positive control, and test specimens

Group	*n*	Mean±SD	df	*F*	*P*	*Post hoc*
Control	30	99.60±0.67	2	1935.14	0.001*	Control > Test > Positive
Positive control	30	44.96±6.33				
Test	30	96.90±1.8				

*Significant <0.05. SD: Standard deviation

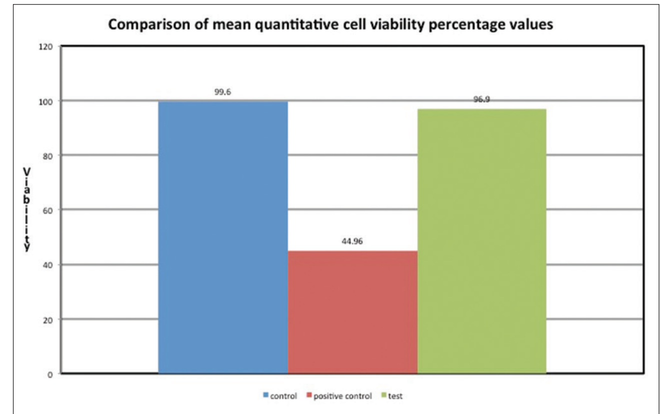

Figure 2: Graphic presentation of the percentage viability of the cells

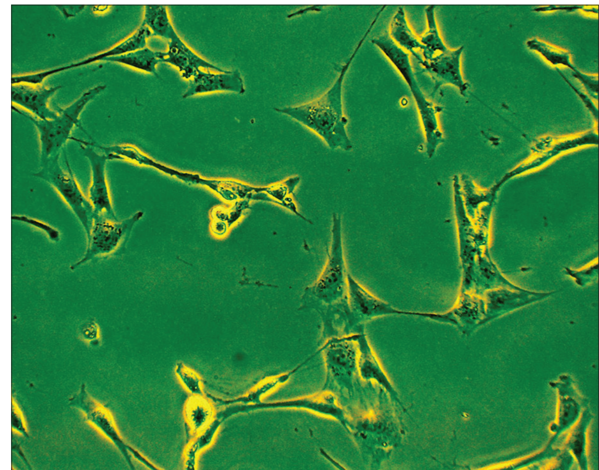

Figure 3: Morphology of the control group cells taken at ×20, showing active proliferation of the fibroblasts cells

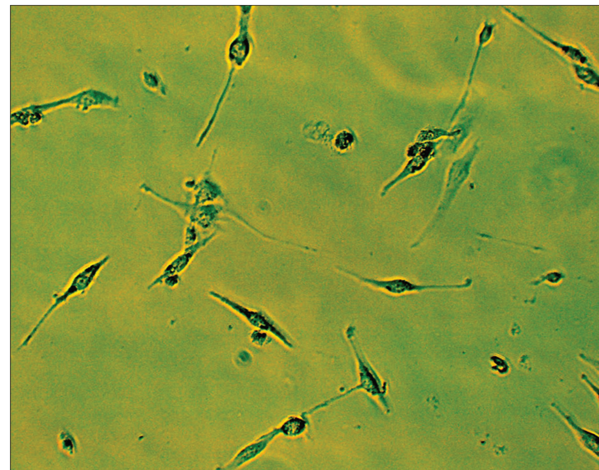

Figure 4: Morphology of the positive control group cells seen at x20, showed necrotic cells.

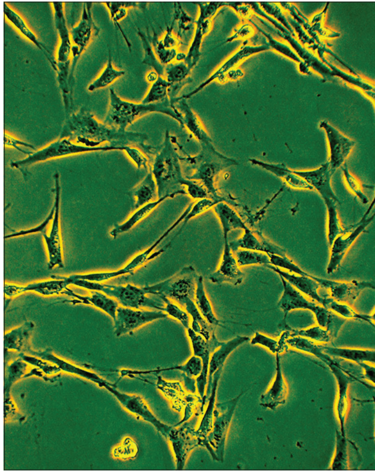

Figure 5: Morphology of the test group cells taken at magnification, showing active proliferation of the fibroblasts cells

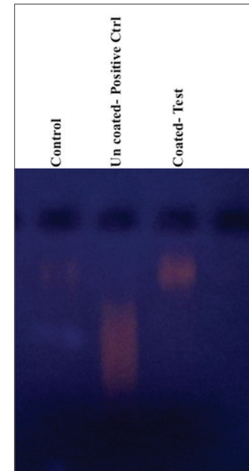

Figure 6: DNA fragmentation assay

in shape indicating apoptosis. The teflon encased magnets showed negligible toxicity. The results of this study were in concurrent with the findings of the study done by Sandler *et al.*[19] They found that the Nd-Fe-B magnets were free from cytotoxic effect on fibroblasts-like cells.

The high toxicity of the positive control group may be attributed to the fact that Nd-Fe-B magnet is highly corrosive in nature. The main metal that may leach out from the magnet would be iron. Haoka *et al.*[20] found high concentration of Fe ions in the corrosion solution used for their study. Thus indicating Fe to have more tendencies to be released from the magnet under corrosion attack and this may cause cytoxicity.

Contradicting results were found in the literature, wherein in an *in vitro* cytotoxicity study conducted with indirect contact method, the coated Nd-Fe-B magnets exhibited toxic effect.[21] However, with neutral red uptake assay using indirect contact method had also shown no toxic effect from coated Nd-Fe-B magnets.[22] Therefore, further investigations may be done to check the cytotoxicity using different materials and methods to ensure safety. This could be the scope for other researchers to take up the study.

Hopp *et al.*[23] showed that Sm-Co magnets had a strong tendency for corrosion and exerted a considerable cytotoxicity. Nd-Fe-B magnets had a lesser tendency for corrosion and exhibited only moderate cytotoxicity. The finding of the above study justifies the validity of the type of magnet chosen for the current study. The above finding in the study by Hopp *et al.* is due to the activity of cobalt in Sm-Co magnets. The fibroblasts cultured in close contact with bare Sm-Co magnet had deleterious effect of the magnet on the cell viability and proliferation as compared to Nd-Fe-B.

In the current study, mouse fibroblasts were used as a cell line model. However, the primary buccal epithelial cells would have been more ideal for this study as the magnets come in close contact with the mucosa of oral cavity. Due to lack of laboratory infrastructure and nonavailability of buccal epithelium cell lines, it was not used. This is one of the limitations in the present study. However, this can be the scope for future research to check the cytotoxicity on human buccal epithelial cell lines.

CONCLUSION

The indigenously encased Nd-Fe-B magnet had no toxic effect on the mouse fibroblasts. Statistically significant differences were seen between positive control group (bare Nd-Fe-B magnets) and the test group (magnets encased in teflon sleeve).

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Conflicts of interest
There are no conflicts of interest.

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