

# Comparison of In-Vitro Conditioning of Two Types of Mandibular Bone Plates in Modified Simulated Body Fluid Characterization with Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy

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## ABSTRACT

**Objectives:** In this study the conditioning of two mandibular mini plates was done in modified simulated body fluid at 37°C.

**Study Design:** An experimental study

**Place and Duration of Study:** This study was conducted at in the laboratory of National University of Science and Technology from March to March 2013.

**Materials and Methods:** The two brands of mini bone plates were selected, one imported from Germany while other was manufactured in local industry. The modified simulated body fluid (m-SBF) was prepared by dissolving reagent in deionised water. The conditioning was done at 1, 7, 14, 21 and 28 days. The surface changes produced after conditioning were analyzed under scanning electron microscope (SEM). The energy dispersive X-ray (EDX) analysis was also used to identify compositional variations of the MPP and MPG surfaces.

**Results:** The SEM revealed almost similar changes in body fluid environment in both plates however the surface of MPG was anodized while no anodization was done on MPPs while in EDX analysis the main elements found were Ti (Titanium) and O (Oxygen) in MPP and MPG. O was more initially then gradually decreases with passage of time in case of MPG while in MPP the O was in low concentration in early conditioning time and then it decreased.

**Conclusions:** The surface of MPG was anodized while no anodization was done on MPPs.

**Key Words:** Scanning electron microscope, bone plate, conditioning, simulated body fluid

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## INTRODUCTION

The fixation of open mandibular fractures is usually done through bone plates.<sup>1</sup> In Pakistan two types of bone plates are used, one imported from abroad and other is manufactured in local industry. All the aspects of both these bone plates have been extensively studied except conditioning in modified stimulated body fluid. In in-vitro conditioning, it is examined what type of changes are produced on the surface of plates when provided with same body temperature, pH and fluid medium outside the body. Scanning electron microscope (SEM) is very useful equipment for analysis of surfaces of materials placed in organic environment. SEM image has higher resolution than optical microscope.<sup>2,3,4</sup> It has multiple benefits such as no complex preparation for studying samples, wide

magnification range. While the disadvantages of SEM are difficult to identify cellular types and sometimes after sample preparation artifacts may appear.<sup>5,6,7</sup> The aim of this study is to compare two types of plates on the bases of periodic conditioning in modified simulated body fluid at body temperature and body pH through SEM.

## MATERIALS AND METHODS

This experimental study was conducted at the laboratory of National University of Science and Technology from March to March 2013.

**Bone plates:** Bone plates used in this study were made of commercially pure titanium of ASTM F67 grade II. Two types of plates were used, Mini Plate Germany (MPG) and Mini Plate Pakistan (MPP). The MPG was supplied by Surgiline Company Berlin, Germany. While the MPP were purchased from Moin International Company Sialkot, Pakistan. The dimensions of plates were 1mm×3mm×6mm.

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**Modified simulated body fluid:** The modified simulated body fluid (m-SBF) was prepared by dissolving reagent in deionised water.<sup>8</sup>

All the reagents and compounds were purchased from Sigma USA. All reagents were first measured on analytical weight balance. The apparatuses (glass beaker, pipette, electronic stirrer and glass flask) were washed with 1.0 M of HCl, neutral detergent, and deionised water. Initially, approximately 700 ml of deionised water were poured into a 1000 ml glass beaker, and this was stirred using an electronic stirrer. The reagents were added one by one after each preceding reagent had completely dissolved (Table 2).

The HEPES was previously dissolved in 100 ml of aqueous 0.2 M NaOH. The solution was adjusted to a pH of 7.4 by titrating aqueous 1.0 M of NaOH into the dissolving solution. The solution was cooled to room temperature (23°C), and the total volume adjusted to 1000 ml by adding deionised water. It was poured into electrochemical cell.

#### Methods

**Sample preparation:** All the apparatus (beakers, silk, scissor and specimens) used in this study were washed thoroughly with distilled water and acetone. All specimens which were six of MPP and six MPG of dimensions (1mm thickness, 3mm width and 6mm length) were tied with silk wire. Silk was used in sterilized form. It was cut into pieces of similar length with sterilized scissor. Six specimens of MPP were suspended with silk in a 250 ml capacity beaker filled with m-SBF and six specimens of MPG were suspended with silk in another 250 ml capacity beaker filled with m-SBF.

**Periodic conditioning:** All the specimens were hanged in middle of beakers so that they would not touch bottom of beaker and all the surfaces of specimens were exposed to the m-SBF. The beakers were placed in water bath (digital constant temperature tank HH-4 China) with temperature settled at 37°C. One sample from each of the beakers was removed at intervals of 1 day, 7<sup>th</sup> day, 14<sup>th</sup> day, 21<sup>st</sup> day and 28<sup>th</sup> day.

**Table No.3: EDX of MPG**

| Element   | 0 day | 1st day | 7th days | 14th days | 21st days | 28th days |
|-----------|-------|---------|----------|-----------|-----------|-----------|
| Carbon    | 0.82  | 0       | 1.82     | 0         | 2.16      | 0         |
| Oxygen    | 22    | 17.27   | 17.19    | 15.21     | 0         | 18.3      |
| Fluoride  | 0     | 0.84    | 0.42     | 1.32      | 0         | 0         |
| Sodium    | 0     | 0.16    | 5.34     | 0         | 5.25      | 0.26      |
| Aluminium | 0     | 0.03    | 0.19     | 0         | 0         | 0         |
| Chloride  | 0     | 0.11    | 3.64     | 0         | 2.64      | 0         |
| Potassium | 0     | 0       | 0        | 0         | 0         | 0         |
| Calcium   | 0     | 0       | 0.03     | 0.11      | 0.02      | 0         |
| Magnesium | 0     | 0       | 0        | 0         | 0.04      | 0         |
| Nitrogen  | 0     | 0       | 0        | 0         | 0         | 0         |
| Titanium  | 76    | 81      | 70.68    | 83        | 89.39     | 81        |

**Scanning electron microscope analysis:** Scanning electron microscopic examination (JSM-6490A, Jeol, Japan) of plates was done to periodically analyze changes in surface topography of bone plates.

## RESULTS

The chemical composition of Ti used is given in Table 1. And composition of modified simulated body fluid is given in Table 2.

**Scanning electron microscope analysis:** In SEM analysis the main elements found were Ti (Titanium) and O (Oxygen) in MPP and MPG (Table 3, 4). O was more initially then gradually decreases with passage of time in case of MPG while MPP the case was reversed. F (Fluoride) appeared on 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> day both in MPP and MPG.

**Table 1: Chemical composition of cp Ti**

| Titanium | nitrogen | Hydrogen | Carbon | iron | Oxygen |
|----------|----------|----------|--------|------|--------|
| Balance  | 0.03     | 0.0125   | 0.08   | 0.30 | 0.25   |

**Table No.2: composition of modified simulated body fluid**

| Compounds                                                | Reagents                                             | Amount  |
|----------------------------------------------------------|------------------------------------------------------|---------|
| Sodium Chloride                                          | NaCl                                                 | 5.403g  |
| Sodium Bicarbonate                                       | NaHCO <sub>3</sub>                                   | 0.504g  |
| Sodium carbonate                                         | Na <sub>2</sub> CO <sub>3</sub>                      | 0.426g  |
| Potassium Chloride                                       | KCl                                                  | 0.225g  |
| Potassium DiHydrogen Phosphate                           | NaH <sub>2</sub> PO <sub>4</sub> . 3H <sub>2</sub> O | 0.230g  |
| Magnesium Chloride hexahydrate                           | MgCl <sub>2</sub> .6H <sub>2</sub> O                 | 0.311g  |
| Sodium hydroxide                                         | 0.2 M—NaOH                                           | 100ml   |
| Calcium Chloride                                         | CaCl <sub>2</sub>                                    | 0.293g  |
| Sodium sulphate                                          | Na <sub>2</sub> SO <sub>4</sub>                      | 0.072g  |
| Sodium hydroxide                                         | 1.0 M—NaOH                                           | 15ml    |
| 2-(4-(2-hydroxyethyl)-1-piperazinyl) ethanesulfonic acid | HEPES                                                | 17.892g |

Carbon was visible in MPG before conditioning, 7<sup>th</sup> day and 21<sup>st</sup> day while in MPP Carbon was absent before conditioning and appeared on 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day. Apart from this sodium, Aluminium, Calcium and Chloride started to appear after 24 hours but on the 28<sup>th</sup>

day only sodium was present in both MPG and MPP. Nitrogen was totally absent in MPG while it was present in MPP in quantity of 0.84 while Mg was appeared on 21<sup>st</sup> day on MPG while it appeared on MPP on 28<sup>th</sup> day.

**Table No.4: EDX of MPP**

| Element   | 0 day | Ist day | 7th days | 14th days | 21st days | 28th days |
|-----------|-------|---------|----------|-----------|-----------|-----------|
| Carbon    | 0     | 0.29    | 0        | 3.74      | 2.45      | 0.21      |
| Oxygen    | 9.73  | 16.14   | 17.27    | 21.38     | 23.91     | 18.15     |
| Fluoride  | 0     | 1.13    | 0.84     | 0.65      | 0         | 0         |
| Sodium    | 0     | 0.09    | 0.16     | 8.77      | 1.85      | 0.42      |
| Aluminium | 0     | 0.06    | 0.03     | 0.26      | 0         | 0         |
| Chloride  | 0     | 0.16    | 0.11     | 7.09      | 0         | 0         |
| Potassium | 0     | 0.14    | 0        | 2.64      | 0         | 0         |
| Calcium   | 0     | 0.18    | 0        | 0.21      | 0         | 0         |
| Nitrogen  | 0     | 0       | 0        | 0         | 0         | 0.84      |
| Magnesium | 0     | 0       | 0        | 0         | 0         | 0.03      |
| Titanium  | 90.27 | 81.77   | 81.37    | 54.16     | 71.79     | 79.27     |

## DISCUSSION

In this study two types (locally manufactured and imported from Germany) of commercially pure titanium mini plates were compared. Energy dispersive X-ray (EDX) analysis was used to identify compositional variations of the MPP and MPG surfaces. SEM analysis indicated no perceptible difference in the surface characteristics of the MPP and MPG at all time intervals. Matthew et al analyzed surface topography of titanium mini plates and screws and found out no considerable changes on EDX analysis up to 24 weeks after implantation.<sup>9</sup>

The difference in surface characteristics of bone plates may also be result of the manufacturing process. The craters, pits, surface cracks and depressions probably arise during production of the sheets from which the miniplates are cut. The fine scratches on the surfaces of all miniplates probably occurred during final polish.

At X1500 magnification, small number of surface irregularities and score marks were visible on the surfaces of MPP and MPG before conditioning. The MPG surface showed on EDX analysis the more quantity of oxygen, which means it, contained large quantity of titanium oxide which makes it more resistant to corrosion.

SEM examination after 24 hours of conditioning (immersion in mSBF at 37°C) showed an organic growth on MPP which was mainly composed of carbon and oxygen with trace amount of sodium, fluoride and Chloride. While MPG revealed no such growth however oxygen concentration decreases from 22% (unconditioned) to 17% after 24 hours examination by EDX analysis.

After 7 days of conditioning, the MPG surface analysis retained same level of oxygen (17%) and quantity of sodium and chloride ions was increased, while in case

of MPP the oxygen concentration was same as that of MPG but sodium and chloride ions were present on surface in lesser quantity as compared to MPG.

The SEM examination of MPP after 14 days of conditioning revealed characteristic flower like appearance with increased oxygen and chloride concentration of 21% and 7% mainly, while MPG showed no chloride and oxygen amount was also less than MPP that is 15%.

After 21 days of conditioning there is more deposition of calcium, sodium and chloride ions on surface of MPG however oxygen quantity was reduced while in case of MPP oxygen concentration was increased and less deposition of sodium ions and revealed no signs of presence of calcium and chloride ions on surface.

EDX analysis after 28 days of conditioning of MPG showed oxygen of 18% and trace amount of sodium salt deposition only while MPP revealed deposition of diverse ions on its surface such as nitrogen and magnesium along with carbon and sodium however oxygen concentration was same as that of MPG. These results were comparable with study done by Balakrishnan et al.<sup>10</sup> He also examined deposition of same ions on surface of commercially pure titanium implants in simulated body fluid.

## CONCLUSION

Surface topography analysis done by SEM in simulated body fluid environment at 37°C and pH revealed almost similar changes in body fluid environment in vitro. The surface of MPG was anodized while no anodization was done on MPPs.

**Recommendations:** The conditioning of bones plates in other types of solutions such as artificial saliva is highly recommended.

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**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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