



In-Vitro Alterations in Biohostability of three commonly used surgical sutures at pH of 5,6,7

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ABSTRACT

Periodontitis is a multifactorial disease, one of the responsible factors include Bacteria present in the oral cavity. Bacteria, like all other organisms, are dependent on the environment for their survival and are influenced by the various host as well as environmental factors for their sustenance. Some of the major environmental factors include nutrition, the presence of oxygen, along with pH, which dictate the nature of the bacteria present as certain bacteria thrive better in acidic environments while others in Alkali environments. In medicine, sutures are extensively used with the aim of post-surgical wound edge approximation or wound healing by the primary union. However, pH is one factor that is constantly changing in the oral cavity due to dietary intake or systemic factors or local accumulation of pus. The present study wished to assess the biohostability of different suture material such as silk, vicryl and chromic gut when pH was changed to 5,6,7 keeping all other environmental factors a constant, including five pieces of sterilised standardised suture lengths & diameter, incubated in pH of 5,6, and 7 exposed to a microbial load of 0.5 McFarland units Lactobacillus subculture in brain heart infusion and incubated at 35 degrees Celsius for four hours to assess the number of bacterial colonies using visual click method. It was found that pH plays a vital role in the Biohostability of suture material as the current study suggests an acidic pH of 5 had more colony-forming units seen among all three suture materials as compared to the other two groups.



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INTRODUCTION

Periodontology has considered Bacteria as the sole cause for infection for centuries and has attributed

several years in the study of different Microflora that comprises the oral Microbiome and the extent of destruction caused by each individual bacteria or bacterial species (Socransky *et al.*, 1984). Newer research over the past 2 decades has provided sufficient evidence of a possible role of both fungi (Slots *et al.*, 1988) and Viruses (Slots, 2004, 2009) as being aetiological agents in the pathogenesis of the Periodontal disease. Despite all these advances, Periodontitis, till date, has been established as a multifactorial disease with multiple predisposing factors and aetiological agents as the severity, pattern and progression of this disease could not be solely explained by the amount of plaque present (Caranza and Newman, 1996). This led to an alternative approach in 1999, where alterations of the Host response towards various pathogens was tar-

geted instead of targeting specific organisms themselves (Golub *et al.*, 1992). The extensive study of different bacteria had suggested various environmental as well as host-related factors that affect the growth of Bacteria at specific sites. They are Temperature, Extent of Oxygen available in the environment, nutrition, and also pH. pH is an abbreviation for the potential of Hydrogen, suggesting the acidic or alkaline nature of a site and the possible change in such a nature that would greatly affect bacteria growth (Yeaw, 1940). There are certain Bacteria that favour an acidic medium while others prefer an alkaline medium optimal for growth. The rationale of the following study was to assess if pH played any significant role in affecting the bacterial growth on different sutures if the Microflora cultured in these materials was standardised. The aim of the present study was to assess the biohostability of different suture materials at different pH and quantify the difference in biohostability using the visual click counter method.

MATERIALS AND METHODS

This study design was initiated after obtaining approval from the scientific and ethical review board of the University. The study design is an In-vitro study with all the facilities and equipment utilized from a microbiology lab attached to a dental college and hospital in Chennai. Three of the most commonly used clinical sutures were selected, namely 4-0 Perma silk, 4-0 vicryl and 4-0 chromic gut, all of which were made by Ethicon manufactured in India by Johnson and Johnson Pvt. They were then standardised by cutting them into 9 equal lengths measuring 1 inch or 2.5 centimetres. These suture materials were then sent for Autoclaving using "Life Stericare 10 L dental autoclave" manufactured by Life Dental and Clinical Autoclaves Pvt. at the specification of 115 degrees Celsius for 15 minutes at 15 bar pressure.

To make a medium for bacterial growth which would not change in pH, 210ml of 10% Phosphate buffer Saline commercially available as a 500 ml container from Lonza Chemical India Pvt. was divided into 3 separate sterile conical flasks obtained from Amar Pvt labelled A, B, C. The pH of A was set at 5 using a combination of both Hydrochloric acid and Sodium hydroxide obtained from the Microbiology and Pathology of a dental college and hospital in Chennai, which was then titrated individually drop by drop till the pH of 5 was attained without overshooting it and matched using pH paper made by Merck. [Figure 1] Using a similar procedure, the pH of B was set till 6, and the pH of C was set till 7. The

following labelled conical flasks were autoclaved at the previously mentioned temperature, time and pressure specifications. After sterilisation, 5 ml were collected from the conical flasks labelled A, B and C and transferred into a sterile plastic container with a lid provided by Amar Pvt. To standardise the Microbiological load exposed 0.5 McFarland units Lactobacillus Subculture was added equally to all 3 containers A, B, and C. Sterile tweezers were used to transfer 3 sutures lengths of 1 inch each from their autoclaved packaging [Figure 2] to all three containers such that there were five each silk sutures, vicryl sutures and chromic gut sutures in container A, B and C. The containers were then closed and placed in an SSU Bacteriological Incubator at 35 degrees Celsius for a period of 4 hours. After 4 hours, using another sterile tweezer, each individual sutures was transferred from their containers to a holding solution of 1ml sterile Normal Saline commercially available as 100 ml mini bottles of 0.9% Sodium Chloride manufactured by the Baxter corporation in sterile Cuvettes labelled from 1 to 27 while carefully recording which number corresponded to which suture kept at which pH and shaken thoroughly. Using a 0.5 ml Eppendorf tube attached to individual tips, normal saline from each cuvette was transferred to separate nutrient agar coated plates labelled A, B and C (as per pH), which were then agitated, and the spread plate method was used to uniformly distribute the suspension across the entire dimension of the plate containing brain heart infusion medium using metal loops sterilised by red hot flame sterilisation and Incubated overnight for the specifications mentioned above.

The subsequent morning the plates were examined to rule out contamination by any other form of Bacterial Colonies and divided into 4 individual quadrants using a permanent marker and the number of individual Colony-forming units was counted for all the plates. [Figure 3]

RESULTS

The following results were obtained from the above-mentioned study are tabulated below based on the average colony-forming units of suture materials at different pH for all five sets of sutures observed by two different Observers to rule out any form of Bias; both observers used visual examination method using a pen and Click counter method to quantify observations. Among the 3 different suture materials, the highest colony forming units were seen at a pH of 5 in all three different suture materials except Vicryl, where the colony-forming units were marginally greater only while comparing pH of

5 and 6. At pH of 6 and 7, the results obtained were variable where vicryl was seen to have lower colony forming units at a pH of 7 while chromic gut had the lowest colony forming units at a pH of 6. Silk was seen to have similar colony-forming units between pH of 6 and 7.

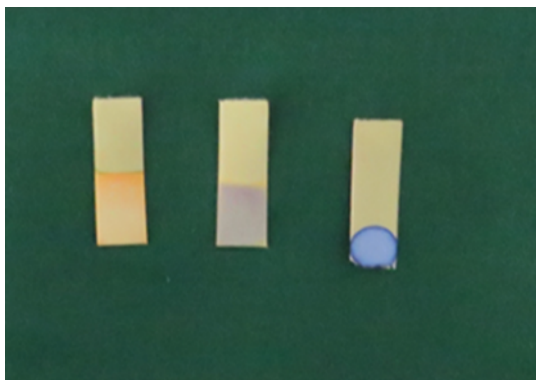


Figure 1: Standardised pH of 5,6,7 utilised confirmed using pH paper manufactured by Merck



Figure 2: Standardised uniform length of sterilised suture materials, each containing 5 pieces of vicryl, chromic gut and silk

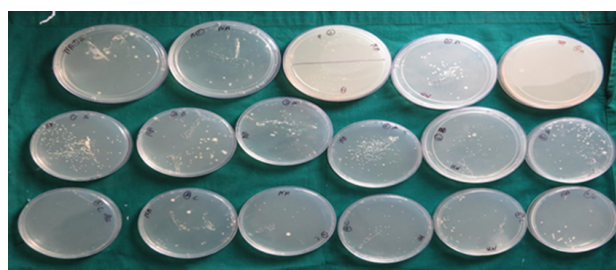


Figure 3: Culture plates corresponding to different pH 5,6,7 and different suture with standardised microbiological load exposed 0.5 McFarland units Lactobacillus Subculture

DISCUSSION

Sutures are routinely used in the clinical practice of periodontology as a means of closing surgical

sites post-surgical intervention allowing healing to occur by either approximation or primary union of gaping wound edges. Initially, they were seen as the only means to close surgical sites; however, newer products such as Antibiotic coated sutures seem to reduce the bacterial load and provide beneficial effects for short periods of time (Soumya *et al.*, 2017). As an alternative to conventional suturing techniques, Cyanoacrylate glues (Mcgraw and Caffesse, 1978) and surgical staples have also gained popularity in performing similar functions. Fibrin based surgical glues currently seem to be the pinnacle of technology in terms of surgical site closure preferred over Cyanoacrylate glues (Bhavsar *et al.*, 2008).

Considering how often sutures, surgical glues and staples have been used in the human body, their biocompatibility and physical properties have extensively been studied. However, all these studies were carried out in an environment where pH was kept constant. The Oral cavity or the mirror of the body is constantly subject to extreme temperatures as well as a drastic change in pH in a matter of seconds for ingestion of certain types of food groups such as Carbonated drinks or Lemon juice pH 2.6, orange juice 3.3, milk 6.8.

The presence of focal infection at a site with an accumulation of pus alters pH, not forgetting certain systemic conditions such as Acid Reflux also drastically alter the pH (Cheng, 2009; Nekoofar *et al.*, 2009). Several studies were performed which evaluated the change in physical properties of suture material as pH was changed, finding both clinical and statistical significance (Tomihata *et al.*, 2001). The possibility of pH significantly altering the physical properties such as tensile stress and modulus of elasticity of suture material lead to the rationale that perhaps it could also alter their biological or microbial properties.

The three pH selected in this study were chosen based on critical pH of Enamel, normal pH of the Oral cavity, changes in pH, which could be expected as a result of the accumulation of pus from any infection as well as changes which occurs due to ingestion of certain types of food such as soft drinks and citrus juices. Despite all these above-mentioned factors, which can change pH, the human body functions in a balanced manner, also known as homeostasis, which is capable of bringing the pH of the mouth back to the physiological pH. Thus, the pH selected for the above study was physiological pH of 7 along with a pH of 5,6. Standardisation and how the entire protocol was formulated and followed was carefully planned and rationed out to ensure

Table 1: Illustrating the mean values of bacterial colony counting using click method among the five samples of three different suture materials exposed to different pH

Different pH	Vicryl (CFU)	Silk (CFU)	Chromic gut (CFU)
pH 5	12	27	24
pH 6	14	10	8
pH 7	3	9	16

best results, including 1 McFarland units of bacterial suspension instead of independent mouth swabs as each successive mouth swab may vary in Bacteria present, which could cause bias. No contamination was seen as a high grade of sterilisation protocol was followed. Among the 3 different suture materials, the highest colony forming units were seen at a pH of 5 in all three different suture materials. A possible explanation behind this could be because of the higher affinity of Lactobacillus colonies towards an acidic pH which favoured their growth in all three sutures irrelevant of their nature (Table 1).

Another important feature that became apparent after the entire study was the nature of the chromic gut, which is preserved in an alcohol-based medium provided by the manufacturer itself. When this seal was broken to standardise the lengths of the suture to be used for the study itself, there would have been some change in the physical properties of the Chromic gut causing it to shrink in size. This could be one of the explanations for the unusual results and observations obtained for the chromic gut; however in previous studies which compared physical properties did not consider the removal from alcohol-based medium relevant enough.

CONCLUSION

In conclusion, the present study showed that Intraoral pH plays a vital role in the biohostability of suture material, where an Acidic pH environment of 5 had more colony-forming units of Lactobacillus subculture among all three suture materials. There is insufficient evidence on alterations in pH and its subsequent effect on intraoral bacterial growth in scientific literature, creating lacunae for the present study. These findings do not warrant a need for immediate change in materials used for suturing or the addition of other products to neutralise the same.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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