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Biocompatibility of Nano-Sized Additives on Flexible Denture Base Material

Ammar Khalid Al- Noori[1], Nadira A. Hatim[2], Dr. Amer A. Taqa[3]

[1]Asst. Prof., Dept. of Prosthetic Dentistry, College of Dentistry, University of Mosul, IRAQ
[2]Professor, Dept. of Prosthetic Dentistry, College of Dentistry, University of Mosul, IRAQ
[3]Professor, Dept. of DBS, College of Dentistry, University of Mosul, IRAQ

Abstract

Aims : To evaluate the biocompatibility of nano – sized additives (Al_2O_3, ZnO and Ag) with different concentrations (0.5 % , 1% and 2%) by Weight to flexible denture base material by mean of cell reaction or histopathological changes of the subcutaneous tissue of the rabbit to implant material .

Materials and Methods : Nine male rabbits were used. Complete anesthesia had been done. Four areas of the rabbit's back were selected,(2 areas on the left side of the vertebral column and 2 areas on the right side). The upper right represented the control specimen, the upper left and the 2 lower represented additive group with 3 concentrations for each additives. The specimens 5mm in diameter and 2.5mm thickness were implanted in subcutaneous area, after sacrificing the animal after 14 days, the biopsy specimen had been excised, histopathological examination were performed by specialist pathologist.

Results: Control and tested groups except 2% Al_2O_3 demonstrated a Grade 2 histopathological changes, while 2% Al_2O_3 demonstrated a Grade 2 to Grade 3 histopathological changes.

Conclusion: The nano-sized additives to flexible denture base material were considered to be biocompatible by mean of histopathological changes.

Keywords: Biocompatibility, Nano-additives, Flexible denture base.

Introduction

Acrylic resin was the most commonly used material for construction of complete and partial denture due to their esthetic value , easy use and ability for relining and rebasing [1,2] .

Thermoplastic material for dental prostheses, Valplast (United state) and flexible (Germany) were related to polyamides group and used for dental applications (Nylon Plastic)[3-5]. Both materials used to create flexible tissue – born partial denture. These materials were not strong enough to be used for conventional tooth born rest seat.The flexibility enhanced patient comfort and affect their satisfaction [4,6].

Flexible denture base material could be considered as an alternative to acrylic resin in the construction of partial and complete dentures, several studies had been performed to evaluate the properties of flexible materials [7-10].

Usage of Flexible denture base material in dentistry has significantly grown in the last decade. As denture based with patient who has sensitivity or allergy with denture polymer. As orthodontic appliance, full and partial dentures [11-13].

Acrylic resin and flexible denture materials had low thermal conductivity and diffusivity compares to metallic denture base which might affect patient acceptance and conformance especially patients wearing complete dentures. The palate was covered by the denture base, the ability to sense transient temperature change at the palate might be affected by the thermal diffusivity of denture base materials and these thermal features of denture base play an impotent role on the gustatory response [14-16].

Polymer had been used in a wide application like denture base materials. It lack the ability to conduct heat, a property required for patient response who wearing a complete a partial dentures to hot or cold applications. Thermal conductive polymer would be needed various kinds of filler, such as metal, metal oxide, ceramic and others have been used to prepare thermally conductive polymer composite[17,18].Addition of metal and fibers improved some physical and mechanical properties of acrylic resin , while the incorporation of silver, copper , and / or aluminum in the form of powder to acrylic resin material improved the thermal conductivity and diffusivity of acrylic resin material [19].

Jasimand Ismail showed that the addition of Al_2O_3 nanoparticles at concentration of (1% , 2% and 3%) by weight to acrylic resin improved thermal conductivity and diffusivity [20].

Rad *et al*., showed that the mean thermal conductivity and compressive strength of acrylic resin reinforced with nano silver were significantly higher than the unmodified acrylic resin, while the tensile strength decreased significantly. Their results suggesting the favorable effect of silver nanoparticles on improving the thermal conductivity of acrylic resin and the use of this material in the palatal area of maxillary acrylic resin dentures was recommended[21].

Biocompatibility could be defined as the ability of the material to perform with an appropriate host response in a specific application [22,23].

In this study, Al_2O_3, ZnO and Ag nanoparticles were chosen as fillers because they not only have higher thermal conductivity, but also have been used in human body and oral cavity in one form or other with out any harmful effect.This study was aimed to evaluate the biocompatibility of nano – sized additives (Al_2O_3, ZnO and Ag) with different concentrations (0.5 % , 1% and 2%) by Weight to flexible denture base material by mean of cell reaction or histopathological changes of the subcutaneous tissue of the rabbit to implant material. This study was approved by the scientific and ethical committee of the college of dentistry, university of mosul.

Materials and Methods

The total number of specimens were 36, the dimension of specimen was 5mm in diameter and 2.5mm thickness, all specimens were made from flexible denture base material.

Five of the Valoplast cartridge were emptied from their granules, and then weighted using electrical balance of 0.0001gm accuracy and the mean of the weights of five cartridge were recorded.Five of the Valoplast cartridge were weighted and the mean of the weights was recorded . from this value the previously recorded mean value of the empty cartridge was subtracted so, the weight of the granules inside the cartridge was recorded. In the present study, Al_2O_3, ZnO and Ag powder nano particles (20-30nm,20-30nm and 80nm respectively) were used with concentration (0.5%, 1% and 2%) by the weight of the Valplast granules were weighted and replaced a side in a plastic tube.

The Valplast cartridge cover was removed then the Valplast was placed on a vibrator, the nanoparticles powder was added gradually in to the cartridge then the cover of the cartridge was closed by knocking the cover of the cartridge. Thecartridge also was shacked very well manually for one minute to ensure that the nanoparticles powder has been distributed uniformly among the granules, followed by placing the cartridge on the vibrator horizontally for two minute, the cartridge was vibrated and rolled manually to avoid accumulation of nanoparticles powder in the cartridge.The furnace was set to 288^{o}C, the heating cylinder which was inserted into the slot present inside the furnace and the furnace was allowed to warm up till it reaches the preset heating which was 288^{o}C. The heating cylinder then removed from the furnace. The Valplast cartridge, the metal disc and finally the short solid metal cylinder inserted into the heating cylinder and left inside the furnace for 16 minutes to allow the granules inside the cartridge to melt. The two halves of the flask were placed in an oven at 65^{O}C for half an hour. Prior to the injection of the denture base material, the two halves of the flask were tightened by the 4 screws securely and returned to the oven, waiting for the granules to melt and to be injected later on[24].The flask removed from the oven and placed inside the injection unit in vertical position in its correct position with the aid of the projection present at the base of the injection unit. In this position the injection opening was to the top surface of the flask.

The heating cylinder together with the Valplast cartridge removed from the furnace by its wooden handle, and placed immediately on the injection opening hole of the flask, and the material was injected inside the flask by the use of the manual injection unit, to give a pressure of 5 bars. After 5 minutes the pressure was released and the flask is removed from the injection unit and allowed to bench cooling to room temperature. The flask then opened and the specimens were removed from the mold. The specimens were smoothed prior to polishing with the smooth blue rubber wheels on the mandrills. The specimens were incubated in distilled water at 37 ± 1 ^{o}C for 48 hours for conditioning.

Nine bred male rabbits, 4-6 months old with an average weight 1250-1350g weight were used,three rabbits for each type of additive, these rabbits were divided in to three groups , each group represent one of the nano sized

additive. This mean that each of the three additives have three rabbits ; on each rabbit four specimens were implanted which represent the three concentrations of the additive, in addition to the control group. The animals were housed in an animal house specially prepared for this purpose,fed in a normal diet and tap water[25-27].

An intramuscular injection of a mixture containing 1.3 ml ketamine hydrochloride (40mg/kg) general anesthetic agent [28], and 0.3 ml xylazine (2mg/kg) sedative, analgesic solution [29]. Complete anesthesia had been obtained within 5 minutes, this dose kept the animal anaesthetized for about 40 minutes.The anaesthetized animal was laid on it's abdomen on the operation board. The fur was shaved in 2 areas of the rabbit's back on the left side of the vertebral column and another 2 area on the right side of the vertebral column.The upper right one represents the control specimen. The upper left one and two lower represents additive groups with three concentration (0.5%, 1%, and 2%). A distance of about 10cm was left between one area and another.The shaved areas of the skin were disinfected using 5% hibitane. Using no.15 detachable blade on a scalpel handle, a small longitudinal incision (about 6mm) was made in the skin of each area, a pocket was created in the subcutaneous layer (by using a blunt dissection) to accommodate the implant, the specimen were applied immediately in to the created pocket, the skin were sutured with one stitch of 3.0 black silk suture.

Immediately after the operation, a mixture of antibiotics containing 2.5ml procaine penicillin (500.000 IU) and 2.5 ml streptomycin (0.5g) had been administrated intramuscularly in the thigh muscle of the rabbit [29]. The same dose were repeated every 12 hours for three days. During this period the animal was isolated from other animals as they will try to harm the animal or remove the suture.

After sacrificing the animal after 14 days, the biopsy specimen had been excised were the sacrificed animal was laid on it's abdomen, then the implantation sites were examined grossly, the implantation and control sites were excised from the skin and kept in 10% formalin for 48 hours, processed in alcohol and xylol embedded in paraffin wax, sectioned at 5 micron thickness, stained with haematoxylin and eosin and examined under light microscope [27 ,30].

Histopathological examination were performed according to Al-Neimee[25] who suggested a system that classify the histopathological changes that could occur during implantation of foreign materials, this system also used by Al-Niami[27]which includes five grades depending upon the severity of the detrimental histopathological changes associated with the implantation of various materials. The grades were as follows:

Grade 1- Only expectable inflammatory and reparative tissue responses have been seen such as infiltration of the area by an inflammatory cells, presence of oedema fluid, fibrin strands , and at late stage formation of fibrous tissue well attached to the original one, no detrimental effects have been seen.

Grade 2- Mild degree of detrimental effects were observed. This grade is characterized by the presence of minimal tissue necrosis around the implanted materials and accumulation of few number of inflammatory cells such as polymorphonuclear and mononuclear cells). Some times newly formed poorly vascularized granulation tissue have been seen.

Grade 3- Moderate degree of tissue necrosis, presence of suppuration, fibrin strands and accumulation of large number of inflammatory cells have been seen. In addition, proliferation of fibrous tissue some times have been observed.

Grade 4- Severe reaction where necrosis, inflammatory reaction around the implanted materials, and inflammatory exudate have been noticed.

Grade 5- Very severe reaction when an extensive amount of tissue nicrosis, very large number of inflammatory cells, and extensive abscessation around the implanted material were noticed.

Specialist pathologist was chosen who had no idea about the materials implanted and had been given the criteria of the grading system, asked him to examine the slides and giving his opinion about the slides' biocompatibility.

Results

Histopathological examination was performed by histopathologist according to (Al –Neimee , 2000)[23] who suggested a system that classify the histopathological changes that could occur during implantation of foreign materials. Fig(1) showed the histopathological changes of implant site of control and (0.5% and 1%) Al_2O_3 groups demonstrated (Grade - 2) histopathological changes while 2% Al_2O_3 demonstrated (Grade -2 to Grade - 3) histopathological changes.

Fig(2) showed the histopathological changes of implant site of control and (0.5% , 1% and 2%) ZnO groups demonstrated (Grade - 2) histopathological changes.

Fig (3) showed the histopathological changes of implant site of control and (0.5%, 1% and 2%). Ag groups demonstrated (Grade - 2) histopathological changes.

Discussion

Biocompatibility could be defined as the ability of the material to perform with an appropriate host response in a specific application [22,23].

Biocompatibility test of the additive materials used in this study were conducted on the basis of cell reaction of the subcutaneous tissue of the rabbit to the implants material as other studies [25,27].

The decision of the specialized pathologist showed that the nano particles additives (Al_2O_3 , ZnO and Ag) at different concentrations except 2% Al_2O_3showed the same grade of histopathological changes as the control group (Grade 2) so the tissue reaction was favorable after additive of nano particles. 2% Al_2O_3 group showed Grade 2 to Grade 3, a minimum degree of deviation of histopathological change from the control group which also could not be considered as a unfavorable tissue changes. Studies demonstrates the use of these nano particles in one from or other in human body and oral cavity without any harmful effect. Nano Ag exhibits potent activity against ocular pathogenic filamentous fungi[31].

Micro-sized and nano-sized Al_2O_3 could be incorporated to acrylic resin to increase thermal conductivity and diffusivity [16,20,32,33].

Nano-sized ZnO was non –toxic and the use of nano (Zno – Tio2) for the synthesis of substituted 1,3,4 – oxadiazole which was considered as novel antibacterial and antifungal drugs[34]. The potential use of nano – ZnO in the food preservation and water purification [35].

Spherical Ag nano particles were synthesized and added to acrylic resin formulation, resulting in successful reduction of adherence of C.albicans . The acrylic resin –silver nanoparticles composite developed was not found to be cytotoxic or genotoxic, according to mitochondrial enzymatic activity, estimation of DNA replication, and non-DNA genomic damage in culture cells. An antifungal biocompatible acrylic resin was developed as a denture base [36].

Conclusions

There were no differences between control and tested groups, all of them showed a mild degree of detrimental histopathological changes and absence of sever inflammatory changes. The nano-sized additives to flexible denture base material were considered to be biocompatible by mean of histopathological changes.

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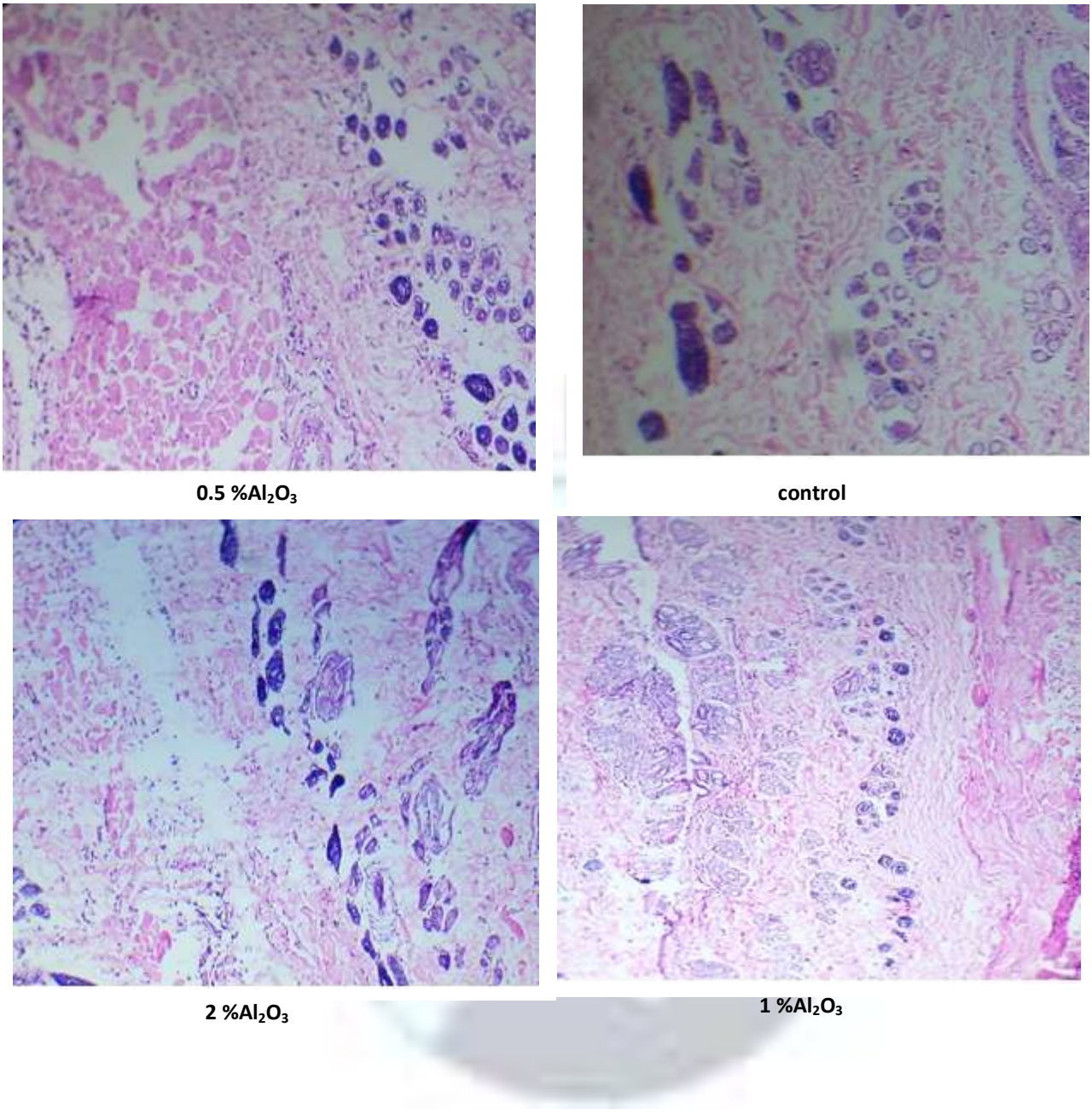

Figure (1): Histopathological changes of implant site of control and Al_2O_3 groups.

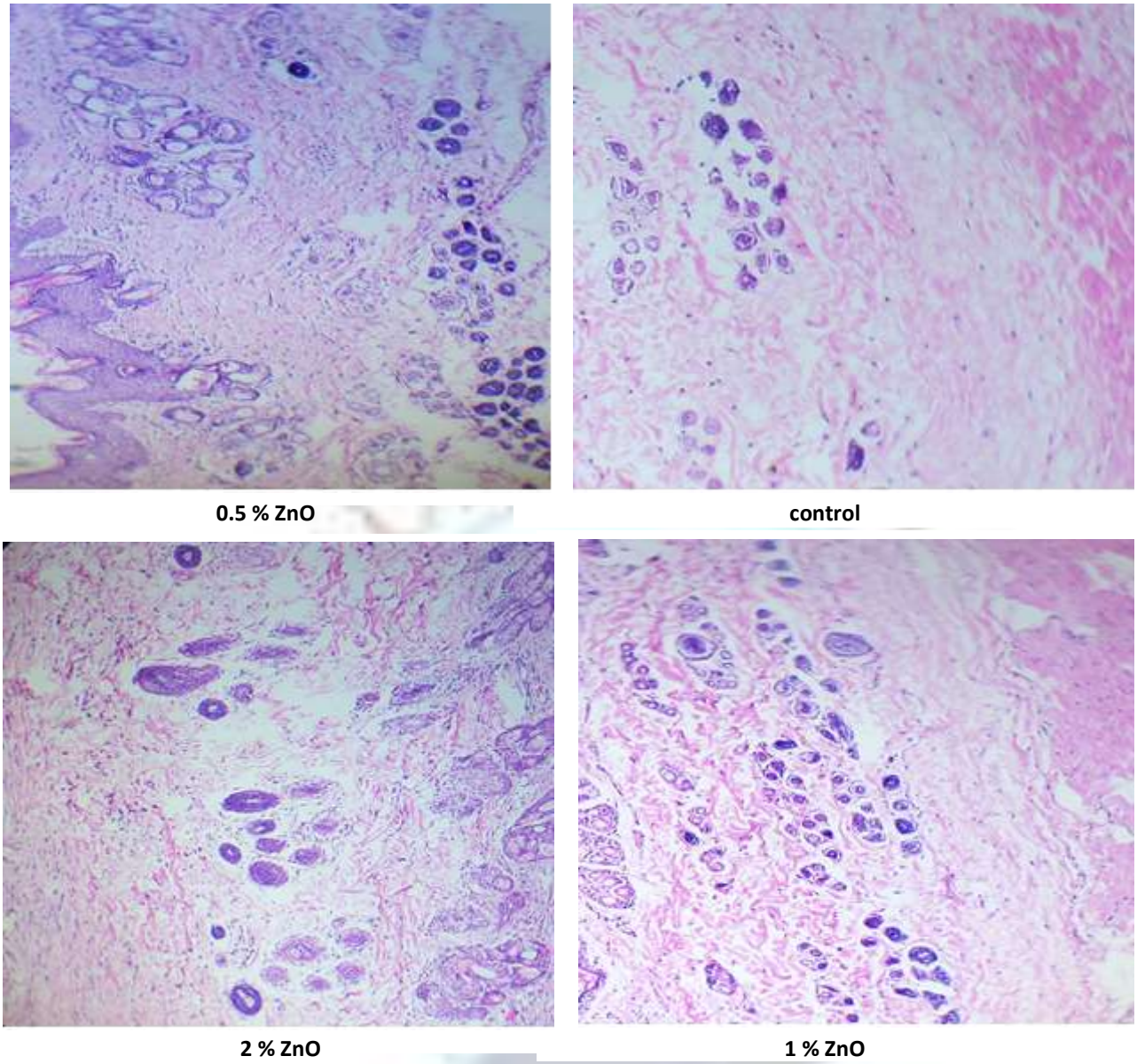

Figure (2): Histopathological changes of implant site of control and ZnO groups.

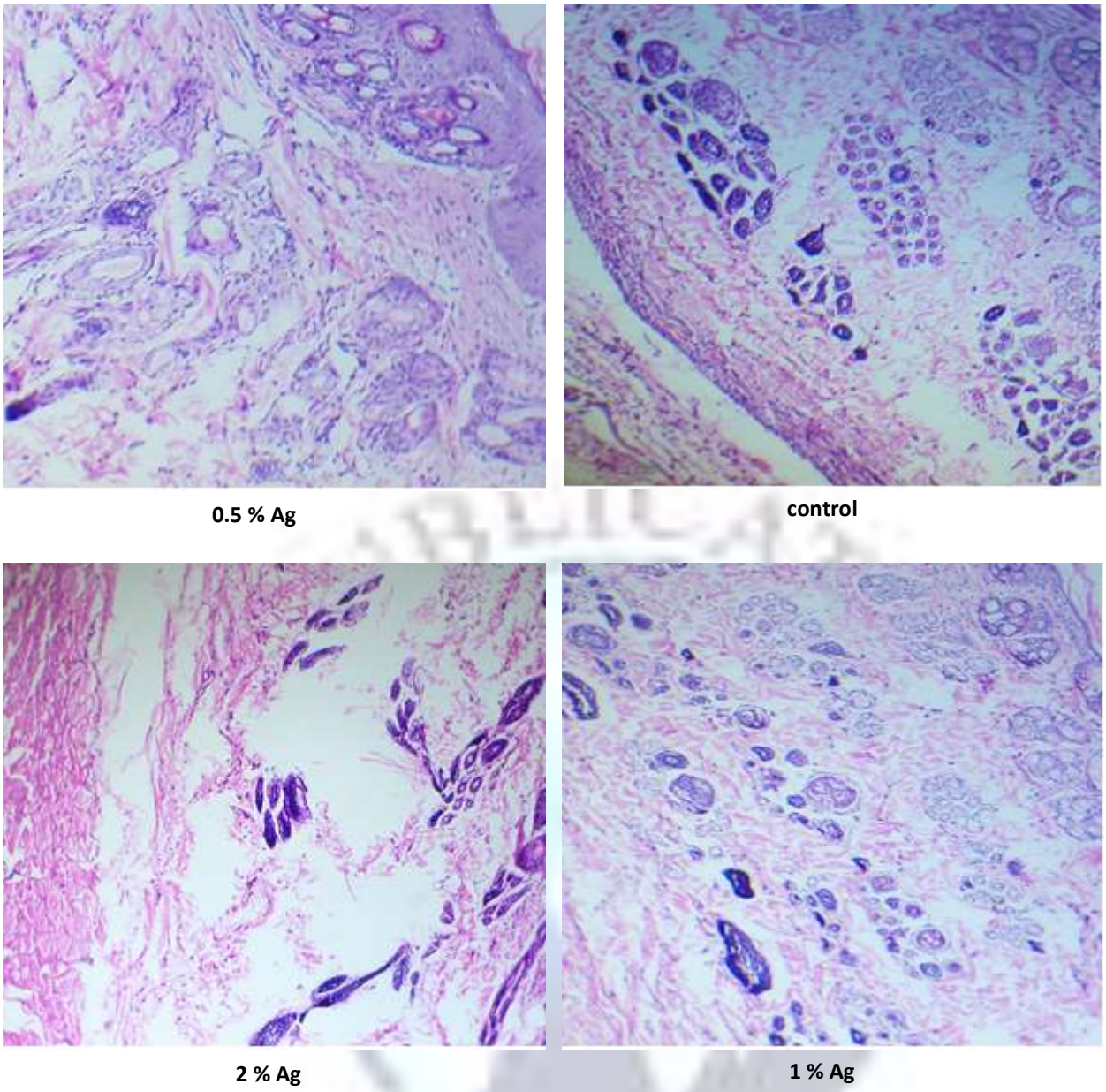

Figure (3): Histopathological changes of implant site of control and Ag groups.