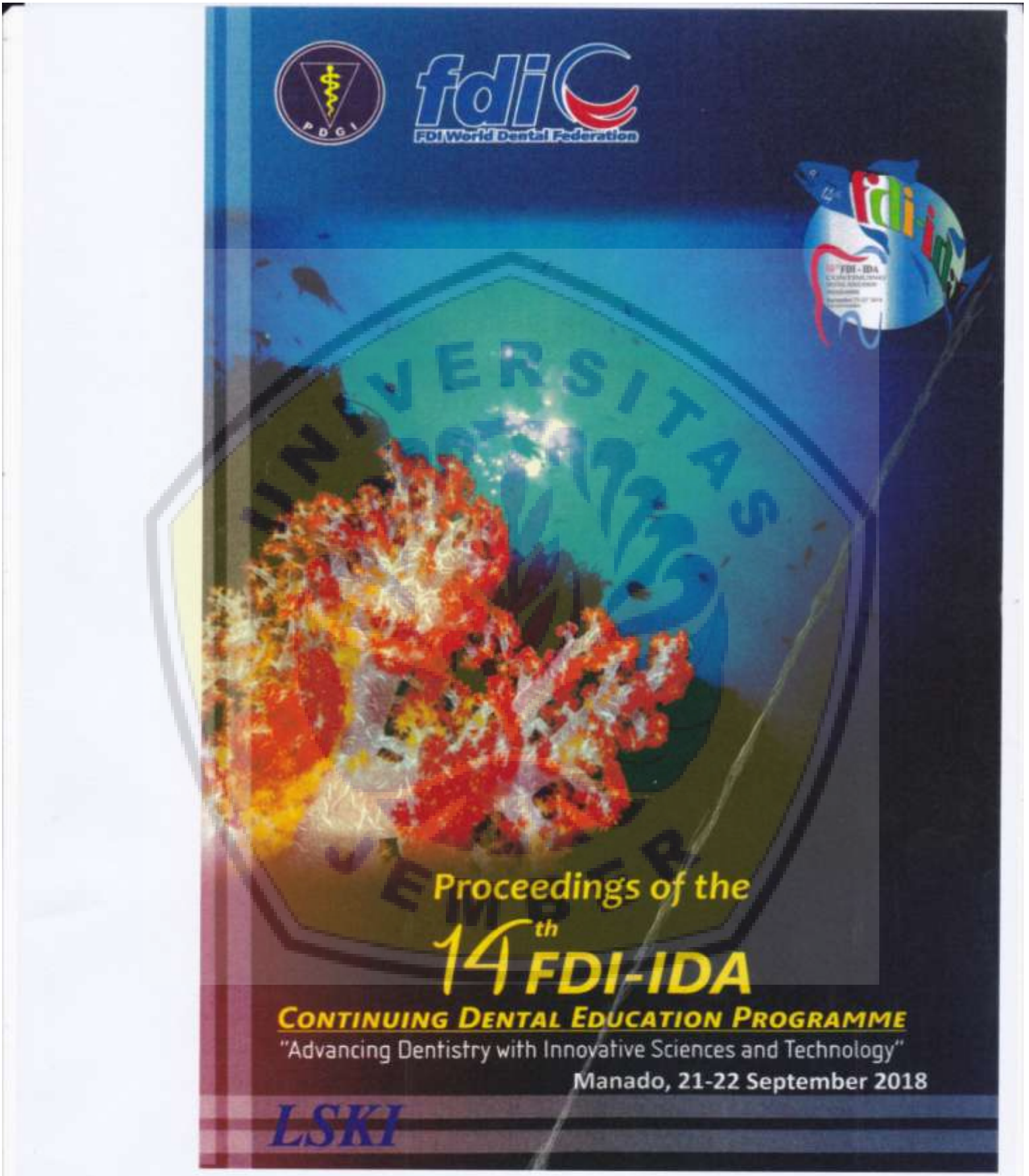

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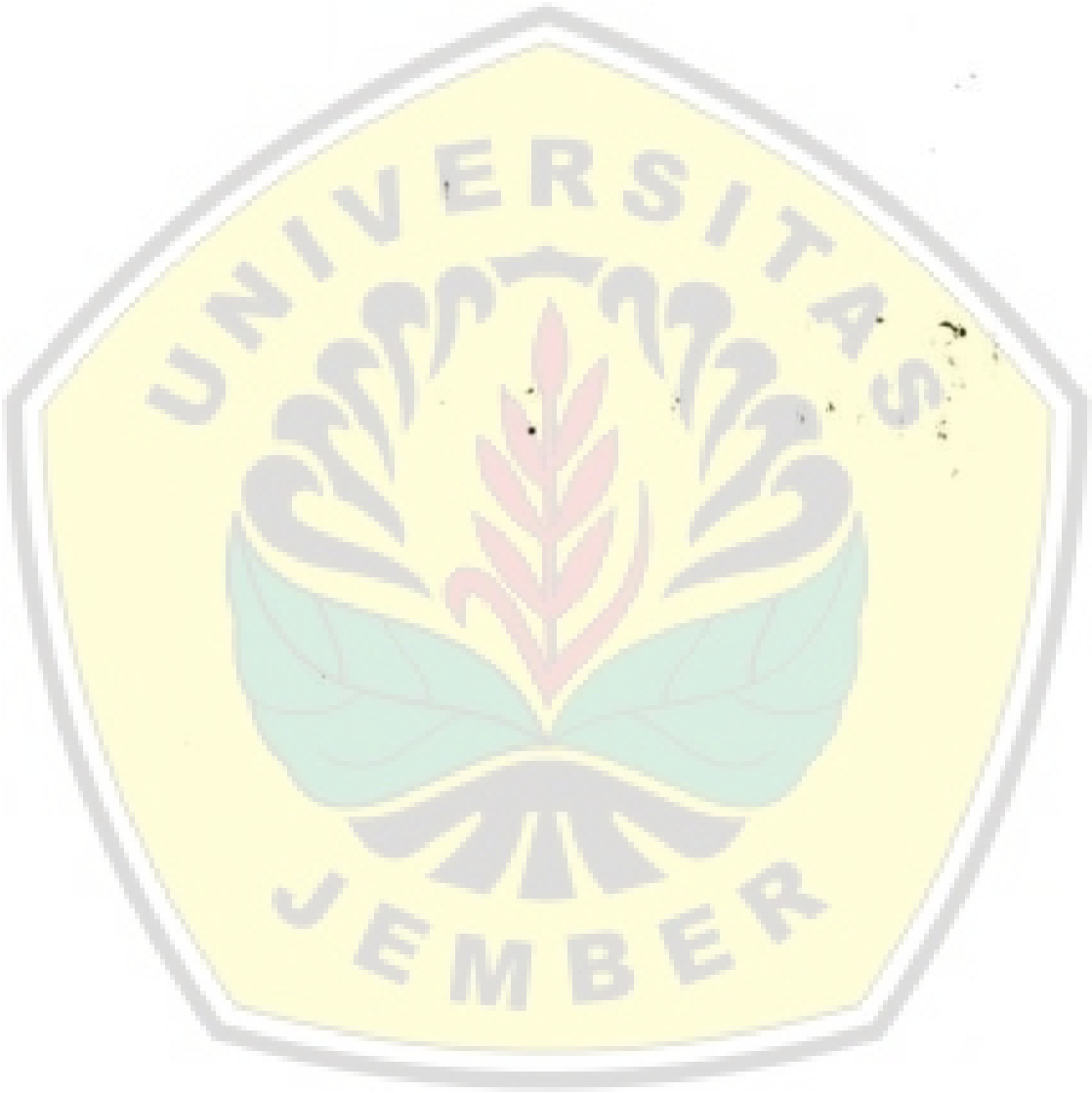

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LSKI

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First published by Lembaga Studi Kesehatan Indonesia (LSKI) Bandung 2018
for 14th FDI-IDA Continuing Dental Education Programme Committee

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ISBN 978-602-60959-4-7

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FOREWORD

Continuing dental education is a lifelong process for dentists who seek excellence in providing the best and current service to their patients. Scientific and technological advances in dentistry has been progressing rapidly in the last few years. Consequently, patients' needs and expectations to receive the highest standard of dental care has also increase.

World Dental Federation (FDI) in conjunction with Indonesian Dental Association hold international scientific meeting and dental exhibition annually. This year, the event will be organized in Manado. It provides a great opportunity for dentists and dental students, in the eastern part of Indonesia especially, to gain knowledge and update their skills.

The theme of this year's meeting is " Advancing Dentistry with Innovative Sciences and Technology" which will enable an international platform for the discussion of the latest findings and future technologies in dentistry.

Chairman,
Sanil Marentek

CONTENTS

RESEARCH

Analysis of Odontoblast-Like Cell Formation After Bioactive Glass Nano Silica Gel of Bagasse Ash Treatment

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Abstract

Introduction: Odontoblast-like cells are reparative dentine former when the original odontoblast cells get apoptotic by irritant. The formation of odontoblast-like cells can be enhanced by applying a material. Synthetic bioactive glass is one of the materials that can be used to induce regenerative dentine formation but this material is difficult to find in Indonesia. The alternative material that can be used is bioactive glass nano silica gel from bagasse ash. This material will form hydroxycarbonate apatite (HCA) layers when contact the body fluid in pulp. The HCA layers will adsorb Transforming Growth Factor-β1 is an important regulator of the proliferation and differentiation of dental papilla mesenchyme cells into odontoblast-like cells. Method: This study was experimental laboratories. Sixteen male wistar rats were divided into two groups, the control group with temporary fillings (caviton) and the treatment group with gel bioactive glass nano silica. Rat teeth were prepared histologically using hematoxylin eosin staining. The number of odontoblast-like cells were counted under a binocular microscope with magnification 400X on three fields of view. Result: The formed odontoblast-like cell in treatment group were more numerous than the control group. Conclusion: bioactive glass nano silica gel can enhance the formation of odontoblast-like cells.

Keywords: Odontoblast-like cell, bioactive glass nano silica gel, bagasse ash

Introduction

Dental pulp is part of a tooth structure in the form of soft tissue which has many blood vessels and located in a room surrounded by hard tissue structures of teeth such as dentine, cementum and enamel [1]. The irritated pulp will repair itself by forming a barrier of mineralized tissue over the surface of the irritated pulp, called tertiary dentin [2]. The process of tertiary dentine formation is reactionary and reparative. The reactionary dentine is produced by surviving odontoblast cells from irritant [3], while the reparative dentine is formed by odontoblast-like cells of mesenchymal cell differentiation of dental papilla in response to replace the odontoblast cells that die from the irritated pulp [4].

The formation of odontoblast-like cells can be enhanced by applying materials placed over the rest of the dentine layer as well as on top of the open pulp as dentine regenerating agents that can adsorb growth factors, especially the Transforming Growth Factor-β1 (TGF-β1) in the pulp to stimulate the formation of odontoblast-like cells [2].

Currently, science and technology in the field of dentistry are continuously developed to improve the deficiencies of existing materials, so as to produce maximum care. One of the major developments in the science of materials is Bioactive Glass [5]. It has a wide range of functions used in the field of health, particularly in the field of dentistry as dentine regeneration material, sensitive dental treatment material, scaffold and implant coating [6]. In general, bioactive glass material contains of Na_2O (Sodium Oxide), CaO (Calcium Oxide), SiO_2 (Silica), and P_2O_5 (Phosphorus Pentoxide). Silica (SiO_2) which is the main composition of

bioactive glass silica material is a material that is easy to obtain, safe, and ideal for application in the field of biomedical [7].

Silica (SiO_2) which is the main composition of bioactive glass can be obtained from natural ingredients such as sugarcane bagasse which has high silica content about 88.13% [8] [9]. Bioactive glass generally has a powder-shaped preparation. Bioactive glass materials have high solubility if they are used as a restoration material. This high solubility can be overcome by the addition of sodium-carboxy methyl cellulose gel base (CMC-Na) [10].

Bioactive glass is now widely developed with nanoparticle size, because in medication, nano size is more effective and easier to diffuse into cells and to stimulate cell repair [11]. The nano-sized bioactive glass particles cause many calcium and sodium ions binding to hydrogen ions which are contained in the body fluids resulting in an increase in local pH and the formation of silanol bonds. The silanol bond groups are subsequently condensed and polymerized to form silica gel onto the surface of the layer to form calcium phosphate and bind to the hydroxyl ions and carbonate ions in the body fluids to form a layer of hydroxycarbonate apatite with a chemical formula ($Ca_{10}(PO_4CO_3)_6(OH)_2$)[12].

The more hydroxycarbonate apatite (HCA) formation will increase the adsorption of the HCA layer to growth factor ie Transforming Growth Factor-β1. TGF-β1 is an important regulator of the proliferation and differentiation of mesenchyme cells located in the pulp to be odontoblast-like cells during formation and regeneration of dentine [13].

The researcher is interested in conducting a further research about the increase formation of odontoblast-like cells in wistar rat pulp (Rattus norvegicus) after gel bioactive glass nano silica treatment from sugarcane bagasse ash.

Methods and Materials

This study used 16 male wistar rats (Rattus norvegicus). The rats were anesthetized intramuscularly with ketamine HCl and Xylazine 0,2ml / 200gr weight of rat prior to preparation. The maxillary first molars were prepared on an occlusal surface using diamond round bur 10 (Edenta, Switzerland) with a depth of ± 1 mm and then perforated using a sonde tip. The rats were divided into 2 treatment groups randomly. In the control group, temporary caviton was applied directly to the cavity. In the treatment group, gel Bioactive Glass was applied to the cavity followed by the temporary caviton.

On day 14 and 28 after treatment (n = 4), the rats were euthanized by decapitation. The upper jaws on the treated molar sections were treated with 10% buffered formalin for 24 hours. The specimens were then decalcificated using 10% formic acid for 1 week. After being soft, the specimens were grown in paraffin, and cut with a thickness of 4-6 µm for the staining of eosin hematoxylin (HE). The odontoblast-like cell cells were observed using a microscope with 400x enlargement in the area of under cavity preparation. Histologic preparations were observed in three different fields of view with three different observers. The observations were taken based on the average number of cells from the observation of three different people.

The obtained data were done a normality test using Shapiro-Wilk test and homogeneity test using Levene Test. After the normality and homogeneity test were done, it was then followed by parametric statistical test, One Way Anova, which then continued with Least Significance Different (LSD) test to know the difference between each research group.

Results

The average number of odontoblast-like cells in each group can be seen in Table 1. The least odontoblast-like cells were found on day 14 after the treatment and the number increased on day 28 (Figure 1).

The result of Shapiro-Wilk test and Levene test with significance value of ($p > 0,05$) showed that the data were normally and homogeneous distributed with significance value of $p > 0,05$. Since the data were normally distributed and homogeneous, parametric statistical

analysis of OneWay ANOVA was then done to determine the differences of all research groups.

Table 1 The average number of odontoblasts-like cells of rats pulp

Groups	Day 14 (X±SD)	Day 28 (X±SD)
Control	20,83±1,290	43,41±2,821
Treatment	27,58±2,603	53,91±2,061

X±SD: the average number of odontoblasts-like cells ± standard deviation

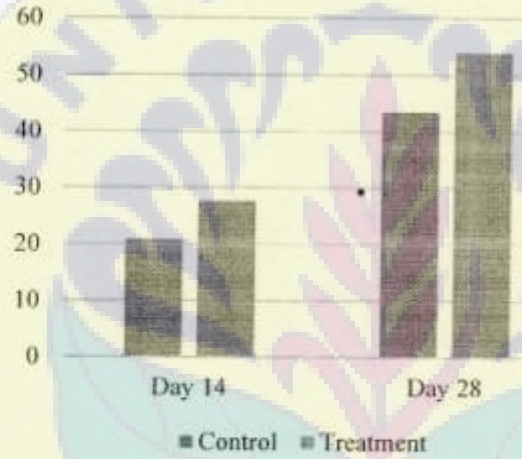
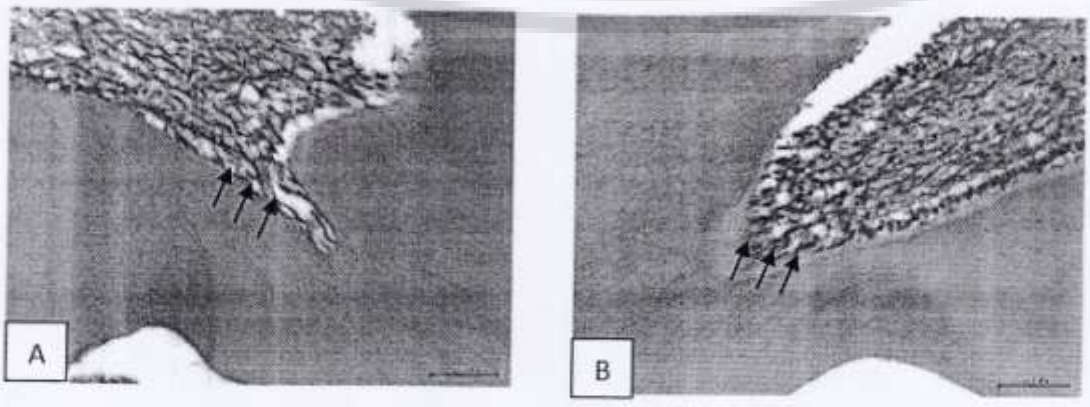


Figure 1 Histogram of difference in the number of odontoblasts-like cells

One Way ANOVA test results showed a significance of 0.000 ($p < 0.05$) which meant there were differences between each group. Furthermore, LSD (Least Significant Difference) post hoc test was conducted to find out the significance of the differences between each group. The result of LSD test showed that there was significant difference between each group with significance value of ($p < 0.05$). This indicated that the group applied by gel bioactive glass nano silica had increased the significant amount of odontoblast-like cells formation. The histologic observation of odontoblast-like cells in the rats pulps with the long columnar shape and thicker cytoplasm were indicated by arrows in Fig. 2.



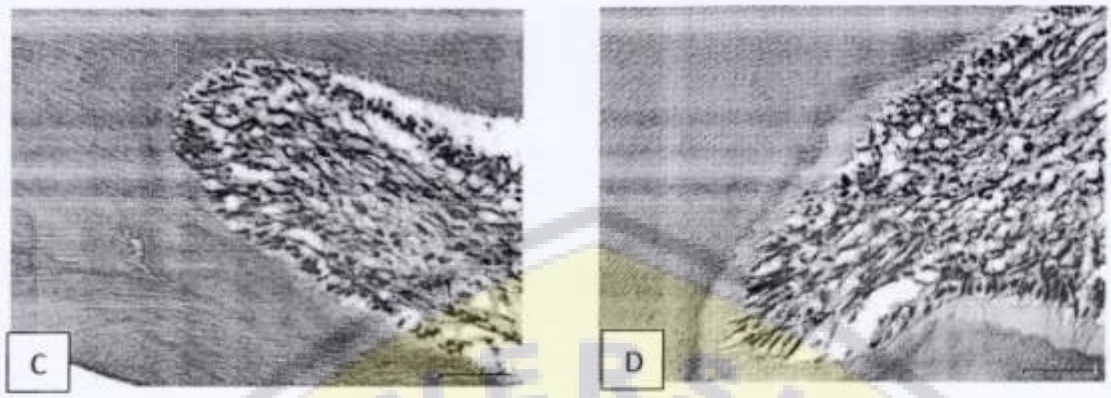


Figure 2. The histological features of the rat's pulp tissue of rattus norvegicus. A. the 14th-day euthanized control group; B. the 14th-day euthanized treatment group; C. the 28th-day euthanized control group; D. the 28th-day euthanized treatment group

Discussion

Odontoblast-like cells are cells in the pulp formed by dental papillary mesenchyme cells differentiated by irritants on the pulp [14]. The process of odontoblast-like cells formation can be accelerated by providing stimulants to the irritated pulp teeth.

Based on the results of the research above, odontoblast-like cells were formed on day 14 and the number was increasing depending on the duration of exposure in each group. This statement is supported by the results study of Babb et al (2017) [15] which states that odontoblasts-like cells begin to form on day 14. Another result of research in vivo also show that odontoblasts-like cells begin to form on day 14 after treatment. [16] The increase in the number of odontoblast-like cells influenced by exposure time is supported by the opinion of Murray (2003) [17] which says that the longer the exposure time, the more it will increase the number of odontoblast-like cells that are formed.

There was significant difference on comparison of control and treatment groups. This was possible because in the treatment group, beside the formation of odontoblast-like cells by physiological processes. It was also influenced by the stimulation of bioactive glass nano silica gel material containing a lot of silica (SiO_2) that can stimulate the formation of odontoblast-like cells. This is supported by the opinion of Subramani et al (2013) [7] which says that silica is the main composition of bioactive glass nano silica.

Bioactive glass nano silica gel material can form silanol bonds when it is contacted with body fluids in the pulp, and there is an increase in pH that can facilitate cell growth. The silanol bond will be condensed and polymerized to form a layer of hydroxycarbonate apatite (HCA). This layer plays an important role in the absorption process of growth factors such as Transforming Growth Factor-β1. This growth factor plays an important role in the proliferation and differentiation of dental papillary mesenchyme cells into odontoblast-like cells. This is supported by the opinion of Wang et al (2014) [13] which states that Transforming Growth Factor-β1 is an important regulator of the proliferation and differentiation of mesenchymal cells to become odontoblasts-like cells.

The formation of odontoblast-like cells in the treatment group was more prevalent than in the control group. This occured because the treatment group treated with gel bioactive glass nano silica occured the release of calcium and sodium ions that would bind to the hydrogen ions in the body fluid of the pulp so that it resulted in an increase in local pH and the formation of silanol bond [18]. An increase in pH will make cell growth better [13]. The formed silanol bonding group will be condensed and polymerized to form silica gel. The calcium ion and phosphate ions contained in the silica gel will come out to form calcium phosphate and bind to the hydroxyl ions and carbonate ions in the body fluids forming the HCA layer ($Ca_{10}(PO_4CO_3)_6(OH)_2$)[19].

Nano-sized particles on bioactive glass nano silica gel will make more bioactive glass materials come into contact with body fluids in the pulp and will increase the formation of HCA layer [11]. The more HCA formation increases the growth factor adsorption ie Transforming Growth Factor-β1 (TGF-β1). This growth factor will bind TGF-β1 receptor that will activate the dental papillary mesenchymal cell cycle to proliferate and differentiate to form odontoblast-like cells [20].

Bioactive glass nano silica gel has been proven in this study being able to stimulate the formation of odontoblast-like cells more than the physiological process. Bioactive glass nano silica from ashes of sugarcane bagasse can be recommended as an alternative material for dentine regeneration. Silica that becomes the main composition of bioactive glass gel nano silica is widely found in Indonesia. One of the silica sources is the waste of sugar cane from the processing of sugar factories that are not maximally utilized [21]. The large potential of waste bagasse can be an opportunity to develop gel materials of bioactive glass nano silica into commodity materials replacing synthetic bioactive glass materials that have not been marketed in Indonesia. Therefore, further research is needed to make bioactive glass nano silica gel be used as an alternative material for dentine regeneration.

Conclusions and Suggestions

Based on the research that has been done, it can be concluded that the gel bioactive glass nano silica from bagasse ash can increase the formation of odontoblast-like cells in the wistar rat tooth pulp (Rattus norvegicus).

Suggestion that can be given is about doing a further research on bioactive glass nano silica gel from clinically justified bagasse ash, and comparing the formation of odontoblast-like cells bioactive glass nano silica gel materials from bagasse ash with materials already used in dentistry practice currently.

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