

## Research Report

## The effect of spirulina gel on fibroblast cell number after wound healing

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### ABSTRACT

**Background:** Wound healing treatment after tooth extraction should be an important consideration due to mouth discomfort and pain. Spirulina (blue green algae) consists of C-phycoyanin,  $\beta$ -carotenoids, vitamin E, zinc, some other trace elements and natural phytochemical which are believed to act as antioxidant and takes part in wound healing process. **Purpose:** The purpose of this study was to examine the effect of spirulina gel on fibroblast cell number after wound healing process. **Methods:** Twenty eight males guinea pig are divided into four group, 7 guinea pig each. They are control group and treatment group which is given 0%, 3%, 6%, and 12% spirulina gel. After tooth extraction, histopathological evaluation was done to count fibroblast cell. The data was analyzed by One-Way ANOVA and Tukey HSD. **Results:** The research has proven the relation between the increased growth of fibroblast cell and spirulina gel application. The higher the doses, the more cell growth. Hence, there has been significant different ( $p < 0.05$ ) among groups. **Conclusion:** Spirulina gel increases the number of fibroblast in wound after tooth extraction and 12% spirulina gel has the most potential ability.

**Key words:** Spirulina gel, fibroblast cell number, wound healing

### ABSTRAK

**Latar Belakang:** Proses penyembuhan luka pasca pencabutan gigi merupakan salah satu hal yang penting karena akan menimbulkan rasa nyeri dan tidak nyaman dalam rongga mulut. Spirulina (Blue green Algae) mengandung C-phycoyanin,  $\beta$ -carotenoids, vitamin E, seng, beberapa trace elemen lainnya, dan phytochemical alami yang terbukti dapat berperan sebagai antioksidan dalam proses penyembuhan luka. **Tujuan:** Tujuan dari penelitian ini adalah untuk mengetahui efek pemberian gel spirulina terhadap jumlah sel fibroblas pada proses penyembuhan luka pasca pencabutan gigi. **Metode:** Dua puluh delapan ekor guinea pig jantan dibagi dalam 7 kelompok, masing-masing terdiri dari 4 ekor. Kelompok tersebut adalah kelompok kontrol dan kelompok perlakuan yang diberikan gel spirulina dengan konsentrasi 0%, 3%, 6%, dan 12%. Setelah pencabutan gigi, dilakukan penghitungan sel fibroblas dengan metode histopatologi. **Hasil:** Penelitian ini menunjukkan adanya hubungan antara pemberian gel spirulina terhadap peningkatan jumlah sel fibroblas. Semakin tinggi dosis gel spirulina akan semakin meningkatkan jumlah sel fibroblas pula. Didapatkan perbedaan yang signifikan antar kelompok ( $p < 0,05$ ). **Kesimpulan:** Gel spirulina meningkatkan jumlah sel fibroblas pada luka bekas pencabutan dan gel spirulina dengan konsentrasi 12% mempunyai kemampuan yang paling potensial.

**Kata kunci:** Gel spirulina, jumlah sel fibroblas, penyembuhan luka

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## INTRODUCTION

Tooth extraction is often done in dentistry. The common reason for tooth extraction is caries teeth with no viable option for treatment, since the decayed teeth can become the source of infection. The other reason is tooth impaction which often cause oral disturbance.

The wound healing process post tooth extraction is one of the problems that clinicians need to concern because the impact is pain and inconvenience inside the mouth cavity. It can cause the disturbance in speech and chewing function, until the healing is completed. There are 11 million people per day suffering pain, inflammation and bruise after having a third molar tooth surgery.<sup>1</sup>

The wound healing process starts a moment after the lesion happens. This process is a complex and systematic series which involves blood cell, tissue, cytokines and growth factor activities.<sup>2</sup> If there is any disturbance in one of these phase, the wound healing process cannot complete optimally or potentially creates new problems such as, bleeding, inflammation, dry socket or infection caused by the microorganism inside the mouth cavity.<sup>1</sup>

Recently, the progress of pharmacy-health technology in the world is focused on the natural ingredient because it is safer to use compares to the drug which contains chemical ingredient. Spirulina contains a lot of nutrition which are useful for the body, such as C-phycoyanin,  $\beta$ -carotenoids, vitamin E, zinc, traced elements and other natural phytochemical. One of the nature ingredients that has been researched and proven to have the capability as an anti-inflammation and anti-oxidant during the wound healing process is C-phycoyanin or blue cell substance.<sup>3</sup>

Spirulina is included in blue green algae group because it contains 14–20% C-phycoyanin. Spirulina is a cyanobacteria class which has the highest blue pigment (C-phycoyanin) compares to other microalgae.<sup>4</sup> Spirulina is rich in mineral and other important substance which are needed by the body, in addition, the usage of spirulina supplement has spread in the society and has many trademark product, such as powder, tablet and capsule.<sup>5</sup>

Laboratory research has been done previously by using the extract concentration spirulina of 3%, 7% and 10% towards the speed of wound healing of rat skin.<sup>6</sup> Those three concentrations show the real result in accelerating the wound healing compares to the untreated sample.

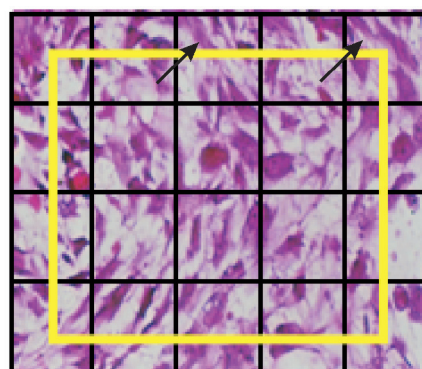
The purpose of this study was to examine the effect of spirulina gel on fibroblast cell number after wound healing process. The amount of fibroblast cell which is viewed histologically becomes the researched variable because the cell has a role for producing collagen fibers needed for accelerating the wound regeneration.

## MATERIALS AND METHODS

The type of researches done is laboratory experimental. The sample used 28 male guinea pigs (*Cavia cabaya*) with

weight of 200–300 gram. Those samples were divided into four groups which had 7 guinea pigs each. They were the controlled group and treatment group which was given 0%, 3%, 6% and 12% spirulina gel. The tooth extraction was done on the mandibular left incisor using the modification of needle holder under 10% ether anesthesia inhaled. Spirulina gel was then applied on the extracted socket of each treatment group. After day five, the mandibular extraction socket was done to the controlled group and treatment group under anesthesia. Post extraction was taken for histological examination by using haematoxylin eosin (HE) painting.

The calculation of fibroblast cell was done by taking the photograph from microscope view after 450 times enlargement.<sup>7</sup> The photo was then given square lines which by then the fibroblast cell around the outer box were counted (Figure 1). This research data was analysed by One-Way ANOVA statistical test and continued with Tukey HSD.<sup>8,9</sup>



**Figure 1.** The calculation of fibroblast cell method (arrow sign) on the surrounding of the outer box.

## RESULTS

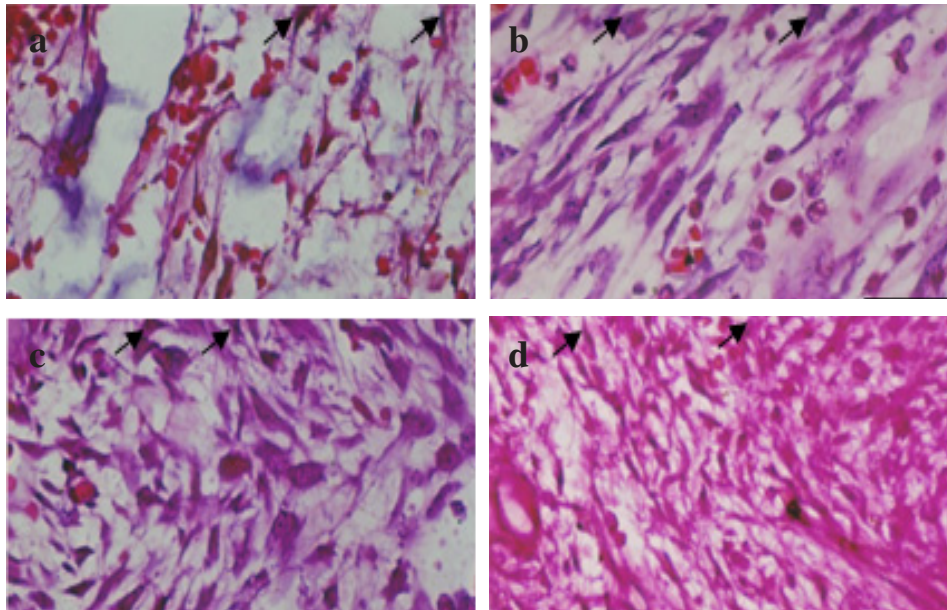
The amount of the highest fibroblast cell is in the sample that is given 12% spirulina gel, meanwhile, the lowest fibroblast cell is in the controlled group.

**Table 1.** Mean and standard deviation of fibroblast cell amount on controlled and treatment group

Group	N	Mean	Standard Deviaton
Controlled	7	14.00*	1.633
3% concentration	7	18.57*	2.440
6% concentration	7	35.57*	2.149
12% concentration	7	39.14*	1.676

Note: \*: significant difference

Before One-Way ANOVA test is done, Kolmogorov-Smirnov statistical test was done and shown that the data is distributed normally and Levene test shows that the data



**Figure 2.** The features of fibroblast cell on each group. a) Controlled group, b) 3% concentration group, c) 6% concentration group, d) 12% concentration group.

was homogeneous. Based One-Way ANOVA test, there was significant value of 0.001 ( $p < 0.05$ ). Therefore the average of fibroblast cell on each group was significantly increased. Table Post Hoc Test from Tukey HSD method showed that there were significant differences on each group comparison.

## DISCUSSION

Guinea pig is chosen as the test animal because it is easy to handle and the socket from the post-extraction has enough width for spirulina gel application. The choice to extract mandibular left incisor is based on the structure and tooth anatomy pattern of guinea pig. Male guinea pig is chosen because it is not affected by hormonal system, therefore, its body is more stable compares to the female guinea pig.

Gel is a semisolid system which contains of suspension made from small inorganic particle or big organic molecule and penetrated by a liquid. The gel is chosen because it is semi solid, soft and elastic so it is easier to apply in the post-extraction socket and can sustain longer in the socket, therefore, it helps the wound healing process. Spirulina gel in this research was made by using CMC Na 3% material as the thickening and stabilizing material. CMC Na is easier to use and it is not influence the function of the condensed substance so it is not affect the result of the research.<sup>10</sup>

The post extraction socket on the controlled group was also applied with gel with 0% concentration to standardize the physical condition of the animal between the controlled and treatment group. 3%, 6% and 12%

spirulina were applied on the wound of post tooth extraction on the guinea pig. This study was done similarly with the previous laboratory research which used 3%, 7%, and 37% concentration spirulina extract on the wound healing of rat skin in which those three concentrations showed the acceleration the of wound healing rather than the controlled group. This test animal is killed on the fifth day because the fibroblast cell is filling the wound area in day 5 to 7.<sup>11</sup>

Generally, the result of the research showed that the average amount of fibroblast cell on the treatment group was increased along with increasing level of concentration of spirulina gel (Table 1). The lowest average amount of fibroblast cell was in the controlled group, while the highest amount was in the test animal which is given 12% spirulina gel concentration (Figure 2).

There were significant differences ( $p < 0.05$ ) on the treatment groups which were given three different concentrations (3%, 6% and 12%) if compared to the controlled group. This condition happened because spirulina gel had a few useful contents such as: C-phycoyanin,  $\beta$ -carotenoids, vitamin E (tocopherols) and zinc to accelerate the wound healing.<sup>11</sup> C-phycoyanin in the spirulina gel is a binding protein in the form of blue pigment which can be used to accelerate wound healing process and anti-oxidant.<sup>3</sup> The previous research showed that the application of C-phycoyanin topically can accelerate the wound healing on the skin.<sup>12</sup>

C-phycoyanin,  $\beta$ -carotenoids, vitamin E (tocopherols) and zinc have the role as anti-oxidant. The inflammation process is marked by implication of multiple inflammation cell such as eusinophil, neutrophil and macrophag which able to produce reactive oxygen species (ROS) which can

delay the wound healing process.<sup>13</sup> The ROS then will be neutralized by the nutrient content, so it has the role to support tissue regeneration such as granulation tissue formation which contains of chronic inflammation cell such as macrophag, limphosit and plasma cell; proliferation of capillary blood vessels and fibroblast and the formation of connective tissue, basalic membrane and matrix between cells and also accelerating wound healing. C-phycoyanin can reduce the functional metabolism activity of neutrophil which cause the slow movement activity. C-phycoyanin can inactivate ROS produced from neutrophil as the mediator of inflammation process.<sup>14</sup>

Healing cascades start when wound happens. The whole process needs an interaction from various cell, including fibroblast. Proliferation and fibroblast migration is important during the healing process.<sup>15</sup> The application of spirulina gel on this research is capable to increase the proliferation and fibroblast cell migration. Proliferation and fibroblast migration is driven significantly by C-phycoyanin of a protein algae. Fibroblas proliferation happens through cyclin-dependent kinase pathway (cdK1 and cdK2), meanwhile the fibroblast migration happens through uPA pathway (urokinase-type plasminogen activator) and then uPA drives fibroblast migration through kemokin pathway (MDC, RANTES, eotaxins, ENA-78) and rho-GTPase protein (Cdc 42 and rac 1).<sup>12,16</sup>

There were significant differences on the treatment group which was given 3% spirulina gel concentration and 6% and 12% concentration. This happened because C-phycoyanin concentration in each preparation was the same with spirulina concentration, therefore, the higher spirulina gel concentration, the increasing amount of fibroblast cell will be higher during the wound healing process. There were also significant differences between the group which was given 6% spirulina gel concentration and 12% concentration. It showed that 12% concentration was the most effective concentration to increase the amount of fibroblast cell on the post extraction wound.

Spirulina which contains C-phycoyanin has low toxicity. On the previous research, the highest concentration of C-phycoyanin (3 grams/kg per oral) was applied on the test animal and it was monitored for 14 days. The result of the research showed that there was no behavioural changes and body weight differences between the treated and the non-treated test animal. The histopathology check is not shown any organ or tissue damages.<sup>3</sup> So, the topical

application of spirulina gel on the post tooth extraction wound is safe without creating side effect.

In conclusion, spirulina gel increase the number of fibroblast and the application of 12% spirulina gel has the potential capability to increase the amount of fibroblast cell on the post extraction wound.

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