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DEVELOPMENT OF A MICROBIAL-BASED GROUTING MATERIAL WITH CALCIUM CARBONATE PRECIPITATES

* Yoko Sakakihara[1], Yoshihiro Kabeyama[2] and Shinichiro Okazaki[3]

[1,2] Graduate school of engineering, Kagawa University, Japan;
[3] Faculty of engineering and design, Kagawa University, Japan,

*Corresponding Author, Received: 15 June 2019, Revised: 19 Nov. 2019, Accepted: 14 Dec. 2019

ABSTRACT: Bio-concrete, which is a concrete with self-repair capability provided by microorganisms, is attracting much attention. In recent years, crack repair grouts based on microbial metabolism have been studied. Because such grouts are flowable aqueous solutions, they differ greatly from grouts based on inorganic or organic compounds and are expected to penetrate into cracks in concrete by capillary action. Therefore, press fitting of bio-grouts is unnecessary. As an additional advantage, these grouts do not impose a load on the environment. In this study, we performed benchtop experiments using conical tubes with a large amount of calcium source. The experimental results show that the addition of a large amount of calcium shows no substantial change in the amount of precipitate. In addition, the amount of precipitated crystals did not substantially differ even when the type of calcium salt was varied. Prolonging the standing time was led to crystal growth, and the amount of precipitates obtained increased. As a result of scanning electron microscopy observations of the crystals obtained under each growth condition, we confirmed that many yeast cells were mixed in the crystals. However, difference was observed in the crystal structure when calcium salt was varied. Moreover, Calcium carbonate formation was observed on concrete as a preliminary experiment for the crack repair of concrete. Crystals were generated even at room temperature, and better results were obtained when the temperature was set to 40 °C.

Keywords: Grout, Calcium carbonate, Calcite, Microbial metabolism

1. INTRODUCTION

Concrete is designed to undergo cracking, and some cracking is considered acceptable to some extent. However, cracking of concrete enables water and carbon dioxide to flow in at the gaps; the concrete becomes carbonated, and the cracks provide channels for the penetration of chloride ions that corrode rebar.

Grouting has been investigated as a crack repair method; however, concerns have arisen about that environmental load imposed by grouting because of the chemical agents involved. Odor at the time of construction has also been mentioned as a problem. Such concerns have spurred recent research into biomaterials, including bio-grouts.

The purpose of this study is to examine the optimum combination for repairing concrete with microbial grout. After in vitro experiments, we used yeast to test whether calcium carbonate was precipitated on concrete. [1-8]

2. IN VITRO EXPERIMENTS

In our recent work, the amount of calcium carbonate generated from bio-grout was examined in a conical tube, and the results were discussed. Based on this, we experimented by adding new conditions.

2.1 Outline of Experiments

The experimental conditions were assembled with reference to the conditions selected from the previous research and the results obtained by simulation last year [1,8]. Table 1 shows the compounding conditions used in our previous study.

Table 1 Formulation of bio-grout as reference.

	Materials	Conc.
Microbe	Yeast	6.0 g/L
Nutrient	D-Glucose	0.40 M
Calcium	Calcium lactate	0.20 M
Buffer	Tris(hydroxymethyl) aminomethane	0.75 M

Note: The pH was set to 9.0, and the experimental temperature was 20 °C.

The simulation results indicated that the precipitation amount of calcium carbonate can be increased by setting the temperature high and preventing a decrease in pH. Yeast is an organism and, as such, has an optimum temperature for growth. Yeast is killed at temperatures greater than 40 °C; thus, the setpoint temperature in these

experiments was 40 °C, and a comparative experiment was performed at room temperature.

We speculated that the amount of precipitated calcium carbonate would increase with increasing amount of calcium salt. We therefore conducted experiments in which the concentration of the calcium salt was varied. In addition, we investigated whether the results differ depending on the calcium salt used in the bio-grout. Table 2 shows the experimental materials. The experimental conditions are summarized in Table 3.

Water (40 ml) as a solvent was placed in a 50 ml conical tube. The buffer and calcium salt were dissolved, the pH was adjusted to 9.0, and the experiment was started by simultaneously dissolving glucose and dry yeast in the solution. Both solutions were covered with silicon, leaving air holes to prevent the evaporation of water.

Table 2 Proposed formulation of bio-grout.

	Material	Conc.
Microbe	Yeast	6.0 g/L
Nutrient	D-Glucose	0.4 M
Calcium	Various	Various
Buffer	Tris(hydroxymethyl) aminomethane	0.75 M

Table 3 Different experimental conditions using the components from Table 2.

	Calcium salt	Conc.
Case 1	Calcium acetate	0.2 M
Case 2	Calcium acetate	0.3 M
Case 3	Calcium acetate	0.4 M
Case 4	Calcium lactate	0.2 M
Case 5	Calcium ascorbate	0.2 M

2.2 Experimental Procedure

To observe the progress of the experiment, we observed the aqueous solution with a microscope on the second day after the start of the experiment to observe whether calcium carbonate crystals had formed.

2.2.1 Microscopic observations

A biological microscope was used for observations. The objective lens was 40×, the eyepiece was 10×, and the image was taken at a magnification of 4× with a digital camera attached to the lens barrel.

An image of the aqueous solution in which calcium lactate was used as the calcium salt is shown in Fig. 1. The small circles are yeast cells, and the darker objects with shadows are crystals. Although the amount of precipitates was small, they could be observed by optical microscopy.

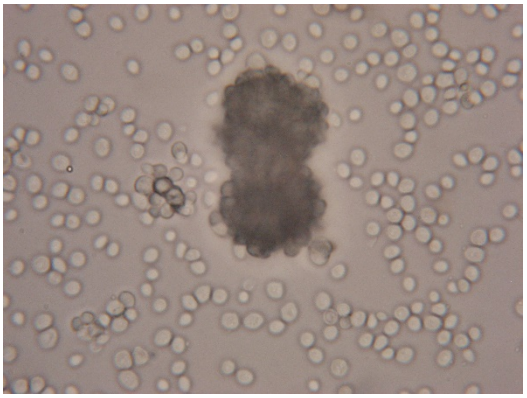

Fig. 1 Microscopic image (magnification: 1600×) corresponding to Case 4 with calcium lactate as the calcium salt.

2.3 Experimental Results

The experiment was conducted for 3 days. The pH change was confirmed every 24 h, and the amount of precipitates was measured. Room temperature was between 24 and 28 °C throughout the experiment.

2.3.1 pH change

The pH of solutions becomes lower as the yeast breathes and releases more carbon dioxide. If the pH becomes lower than about 6, the precipitated calcium carbonate crystals will be re-dissolved. [7] A buffer was added to prevent this, and as a result, there was no significant change in pH. However, the decrease in pH at 40 °C was slightly greater than at room temperature. We attributed this lower pH to the enhanced activity of the yeast at the higher temperature, which caused it to breathe more and discharge more carbon dioxide than the yeast at room temperature.

2.3.2 Measurement of precipitation amount

The precipitate generated in the experiment was collected by vacuum filtration using filter paper (No. 5C). The amounts of precipitates obtained from the samples maintained at room temperature and from those maintained at 40 °C are shown in Fig. 2 and Fig. 3, respectively. Even when no crystal was observed on the filter paper, approximately 0.1 g of material was recovered. We speculated that approximately 0.1 g of yeast remained on the filter paper. In Case 1 (Fig. 3), the amount of precipitates was less at 72 hours than at 48 hours, which is considered to be an experimental error. We consider

the experimental error here is random error, caused by environmental conditions or other unpredictable factors.

In general, the results of this study show that the addition of a large amount of calcium salt did not lead to a large amount of precipitate. This outcome is attributable to two possible causes. First, the experimental period may have been too short for appreciable precipitate growth. Second, the concentration and, therefore, the osmotic pressure may have been too high for the yeast to survive. These possibilities are currently being investigated further. The addition of calcium ascorbate slightly increased the amount of precipitate compared to the other cases. In addition, ascorbic acid was oxidized immediately upon exposure to air, and the resulting crystals were yellow.

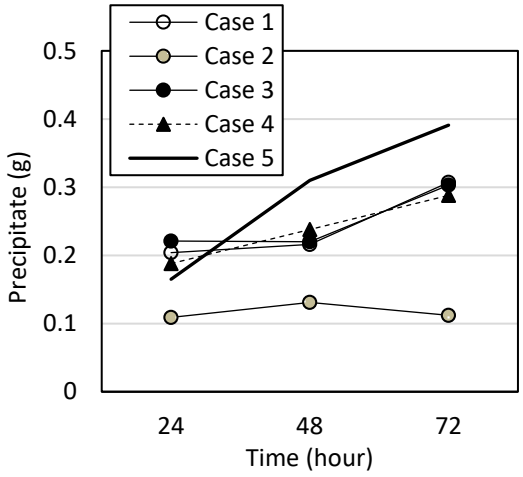

Fig.2 Amount of precipitates collected from samples maintained at room temperature.

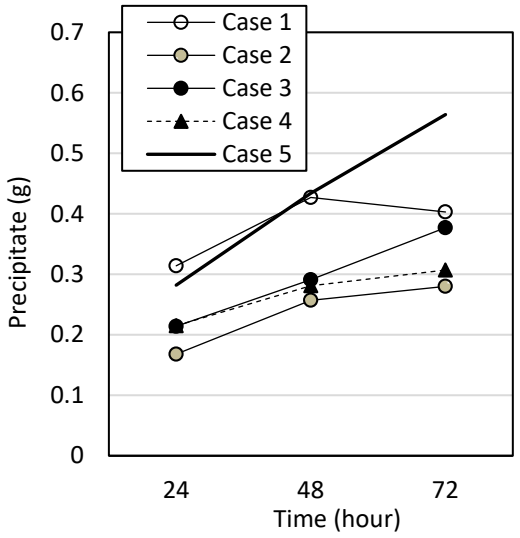

Fig.3 Amount of precipitates collected from the samples placed in a constant-temperature bath at 40 °C.

2.3.3 XRD analysis

The crystal structure of the precipitate obtained in the experiment was analyzed by XRD. The results, presented in Fig. 4, show that the precipitated crystals are mainly composed of calcite.

In Cases 1-3 and 4, in addition to calcite, vaterite was detected. In Case 5 to which calcium ascorbate was added, only calcite was precipitated.

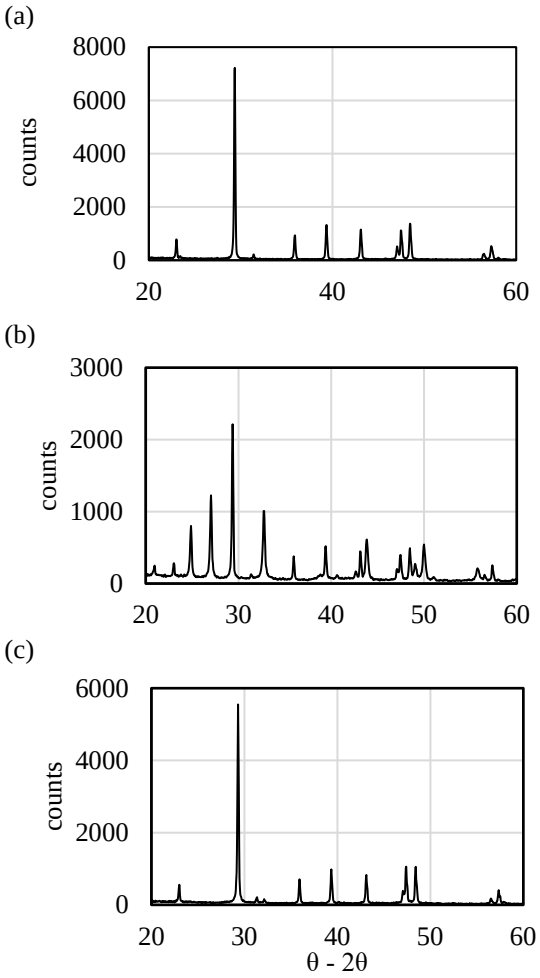

Fig.4 XRD patterns: (a) Calcite; (b) Case 1: 0.2M Calcium acetate; (c) Case 5: 0.2M Calcium ascorbate

2.3.4 SEM analysis

The crystals obtained under each condition were observed by FESEM, and SEM. The images confirmed that many yeast cells were mixed with each crystal. The crystals themselves were spherical. It is speculated that this is because the crystal takes on a vaterite structure at the formation stage. The SEM images of the precipitates corresponding to Cases 1 and 3, where numerous crystals were obtained, are shown in Fig. 5.

Although experiments were conducted in two

systems (one at room temperature and the other in a constant-temperature bath at 40 °C) and the amounts of precipitates differed, no significant difference was found in the crystal structures of the precipitates.

In Case 5 to which calcium ascorbate was added, a peak of only calcite was observed in the XRD, however the SEM image confirmed crystals that might be aragonite. And, the amount is very small. From the SEM image, it was found that many cavities were found in the crystal, and it took longer standing times for crystal growth.

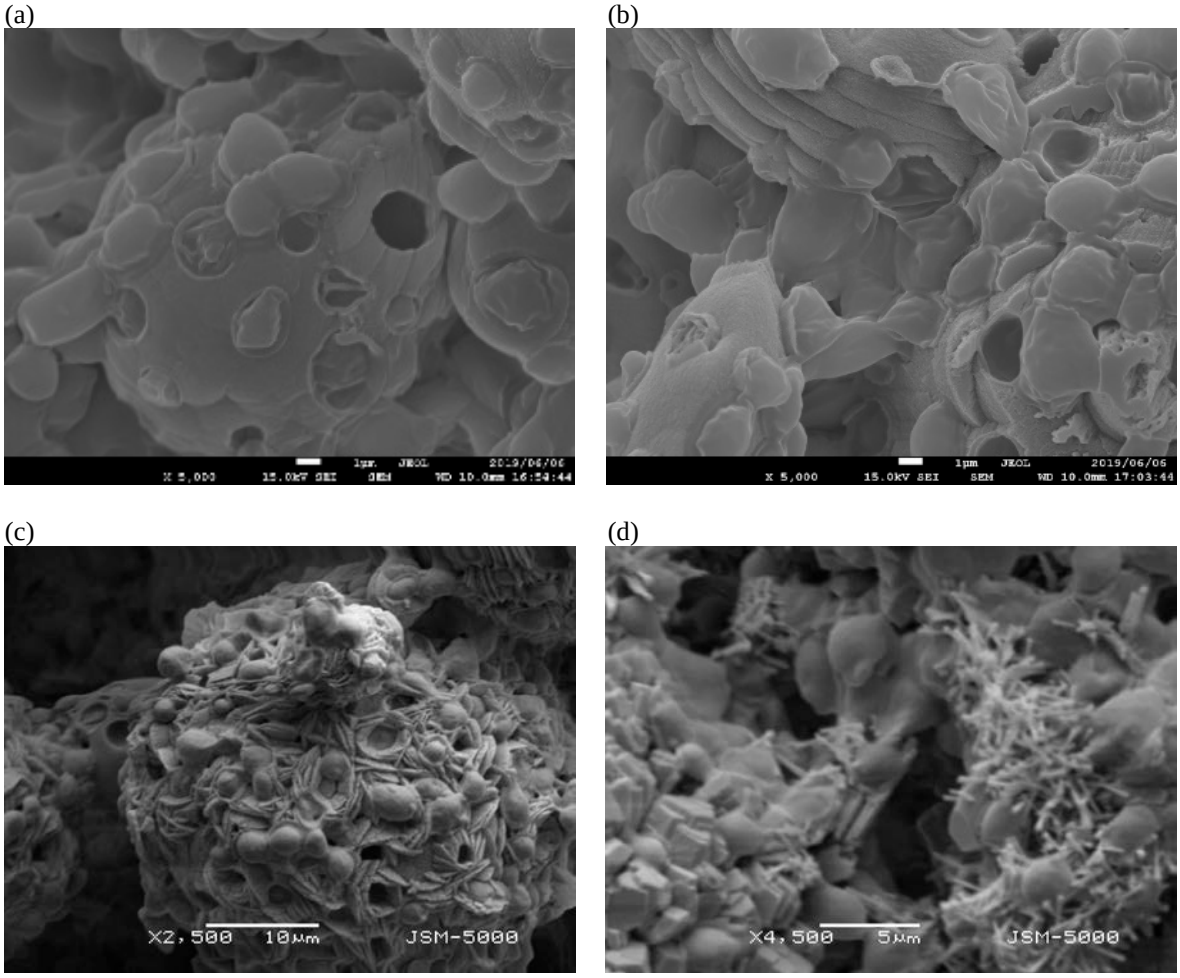

Fig.5 FESEM, and SEM micrographs: (a) Case 1, room temperature (5000×); (b) Case 1, 40°C (5000×); (c) Case 3, 40 °C (2500×); (d) Case 5, 40 °C (4500×).

3. ADAPTATION TO CONCRETE

In the past researches, since concrete is alkaline, bacteria which can grow even under basic conditions have been mainly selected.[9] However, yeast is a bacterium that grows under acidic conditions, and its optimum pH is said to be about 5.[10] Therefore, we investigated whether it was possible to grow under such conditions, and whether calcium carbonate was precipitated.

In general, appropriate knowledge is required to isolate and culture bacteria. In that respect, yeast can be easily grown even by nonprofessionals. Moreover, yeast is cheap and easily available anywhere in the world. In addition, environmental burden is low because it is a resident-bacteria.

3.1 Preliminary Experiment

Before repairing cracks using bio-grout, to understand the growth conditions of the yeast and the generation of crystals, we conducted experiments on the concrete. A silicon was placed as a wall on top of a 10 cm × 10 cm mortar specimen, and the grout was poured into it. The selected grout was that of Case 1. The results of Fig. 2 and 3 show that the best yield is obtained when ascorbic acid calcium is added.

However, this compound is three times more expensive than calcium acetate. When considering the cost, case 1 was the optimal combination.

After the start of the experiment, only water was added as necessary to prevent dehydration. The concreate was covered with a wrap to prevent excessive drying. Two specimens were prepared; one was placed indoors, and the other was placed in a thermostatic chamber at 40 °C.

3.2 Experiment Result Day 2

Crystal growth was checked 2 days after the start of the experiment. A microscope was used; the magnification was 1600×. As a result of microscopic observation, crystals were suspended on the water surface of a solution placed in concrete installed at room temperature. The solution immediately above the concrete was also collected and observed. However, mostly yeast cells were observed, with very few crystals.

3.3 Experimental Result 5th Day

3.3.1 Microscopy

Five days after the start of the experiment, the solution was removed and the concrete surface was observed. A microscope was used for the observation; the images were collected at approximately 200× magnification. Pure calcium carbonate crystals appeared on the concrete surface in the specimen maintained at room temperature (Fig. 6 (a)). These crystals were observed over the whole concrete surface.

For the sample maintained at 40 °C, the crystals apparently aggregated into large flocks (Fig. 6 (b)). An image of the side not in contact with the aqueous solution is shown in Fig. 6 (c). This image was a countless number of small holes on the concrete surface, the inside of the holes was empty and the surface was flat. For the sample maintained at 40 °C, the locations of the crystals on the concrete surface were difficult to determine; however, the crystals clearly grew large, as revealed by observation of the interior of the holes (Fig. 6 (d)).

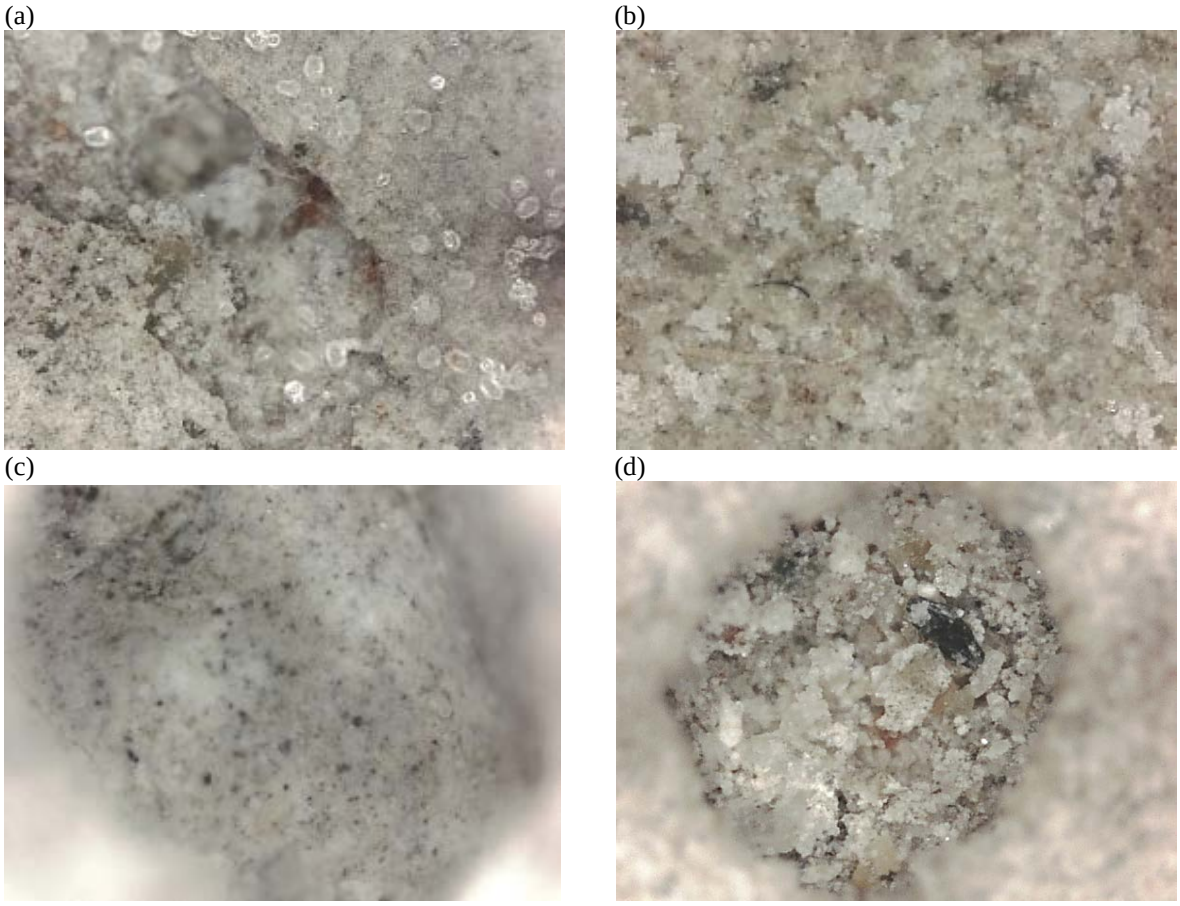

Fig.6 Microscopic images (200×): (a) crystals generated on the concrete surface at room temperature; (b) crystals generated on the concrete surface at 40 °C; (c) a hole in an untested concrete surface; and (d) crystals formed in the hole at 40 °C

3.3.2 Laser Raman spectroscopy

When the crystal which precipitated on concrete was analyzed by laser Raman spectroscopy, the peak of calcium carbonate was confirmed. The image of the Raman spectrum is shown in the Fig. 7.

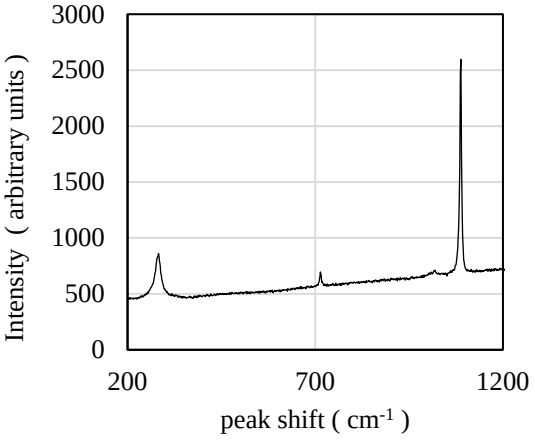

Fig.7 Raman spectrum of precipitates

4. CONCLUSIONS

In in vitro experiments, we expected that a large amount of calcium carbonate could be obtained by adding a large amount of calcium salt; however, no change was observed. Experiments confirmed that the amount of calcium carbonate obtained was almost the same irrespective of the type of calcium salt. Prolonging the standing time led to crystal growth, and the amount of precipitates increased. SEM observations of the crystals obtained under each condition confirmed that many yeast cells were mixed with the crystals. Difference was observed in the crystal structure when calcium salt was varied.

A preliminary experiment was carried out to adapt the bio-grout to concrete, and the process was observed via optical microscopy, and the precipitates were observed via microscope and laser Raman spectrophotometer. The precipitating calcium carbonate on the concrete surface could be confirmed. Crystals were generated even at room temperature, and better results were obtained in experiments conducted at 40 °C.

In the future, we will conduct more practical experiments for crack repair, repeat follow-up observations, and further consider optimal combinations of grout materials.

5. ACKNOWLEDGMENTS

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