

Efficacy of Red or Infrared Light-Emitting Diodes in a Mouse Model of *Propionibacterium acnes*-Induced Inflammation

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Background: Laser/light-based devices may provide an alternative to conventional acne therapeutics in some patients with nonresponsive acne. **Objective:** We investigated the efficacy of red or infrared light-emitting diode (LED) devices in a mouse model of *Propionibacterium acnes*-induced inflammation through clinical examination and histopathological and immunohistochemical studies. **Methods:** A human-derived *Propionibacterium acnes* suspension (10^9 colony-forming units / μ l) was injected into the back of an HR-1 mouse. Then, a 28.9 J/cm² 650-nm red LED or 9.3 J/cm² 830-nm infrared LED was applied to the mouse with *P. acnes*-induced inflammation once daily for 2 weeks. Two weeks after treatment, histological findings with hematoxylin and eosin staining and expression levels of inflammatory biomarkers (integrin α 6, neutrophils, interleukin [IL]-1 β , and matrix metalloproteinase [MMP]-2/9) were evaluated in tissue specimens using immunohistochemical staining. **Results:** Mice treated with red and infrared LED showed clinical improvement in inflammatory nodules compared to mice in the control group. Red LED was much more effective than infrared LED. Epidermal hyperplasia, comedone-like cysts, and integrin α 6 expression improved to a similar extent in the red and infrared LED treatment groups and control

group. Neutrophil, IL-1 β , MMP-2, and MMP-9 expression after treatment with red and infrared LED decreased considerably compared to expression in the control group. **Conclusion:** In a mouse model of *P. acnes*-induced inflammatory nodules, red and infrared LED devices may be an alternative to conventional acne therapies. In addition, a mouse model of *P. acnes*-induced inflammatory nodules is helpful for laboratory research of acne. (**Ann Dermatol** 28(2) 186~191, 2016)

-Keywords-

Acne, Light, Mice, *Propionibacterium acnes*

INTRODUCTION

Acne is a very common skin disorder occurring in the follicular infundibulum^{1,2}. *Propionibacterium acnes* colonization in the follicular infundibulum is a major cause of acne and plays an important role in inducing an inflammatory event³. In addition, *P. acnes* can cause abnormal differentiation and proliferation in epidermal keratinocytes¹. Therefore, acne animal models can be established using *P. acnes*. The establishment of animal models for acne is helpful to expand research fields and to develop new therapeutic modalities. Although various animal models for acne such as the Mexican hairless dog and the Rhino mouse exist, an elucidative model is still needed⁴. Our animal model of inflammatory events was established with the injection of *P. acnes* into the back of an HR-1 mouse.

Laser/light-based devices may provide an alternative to conventional acne therapeutics in some patients. Many types of laser/light devices have been introduced to improve or cure inflammatory acne. Light-emitting diode

Received March 2, 2015, Revised June 25, 2015, Accepted for publication June 29, 2015

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(LED) devices are one of the most popular light-based devices for the treatment of acne. LED devices, especially those emitting blue and red light, directly or indirectly target *P. acnes*⁵.

In this study, we investigated the efficacy of red or infrared LED devices in a mouse model of *P. acnes*-induced inflammation through clinical examination, pathology, and immunohistochemical studies.

MATERIALS AND METHODS

Preparation of the mouse model with injection of *Propionibacterium acnes*

Six-week-old female Hos:HR-1 mice (HR-1; SLC Inc., Hamamatsu, Japan) were kept under conventional laboratory conditions (at 20°C~24°C in a humidified atmosphere of 40%~60%) after 1 week of acclimation. *P. acnes* (ATCC 11828) were isolated from the pustular lesions of Korean patients with moderate inflammatory acne. *P. acnes* from post-log phase cultures were grown on brain heart infusion agar and harvested, heat killed (95°C, 5

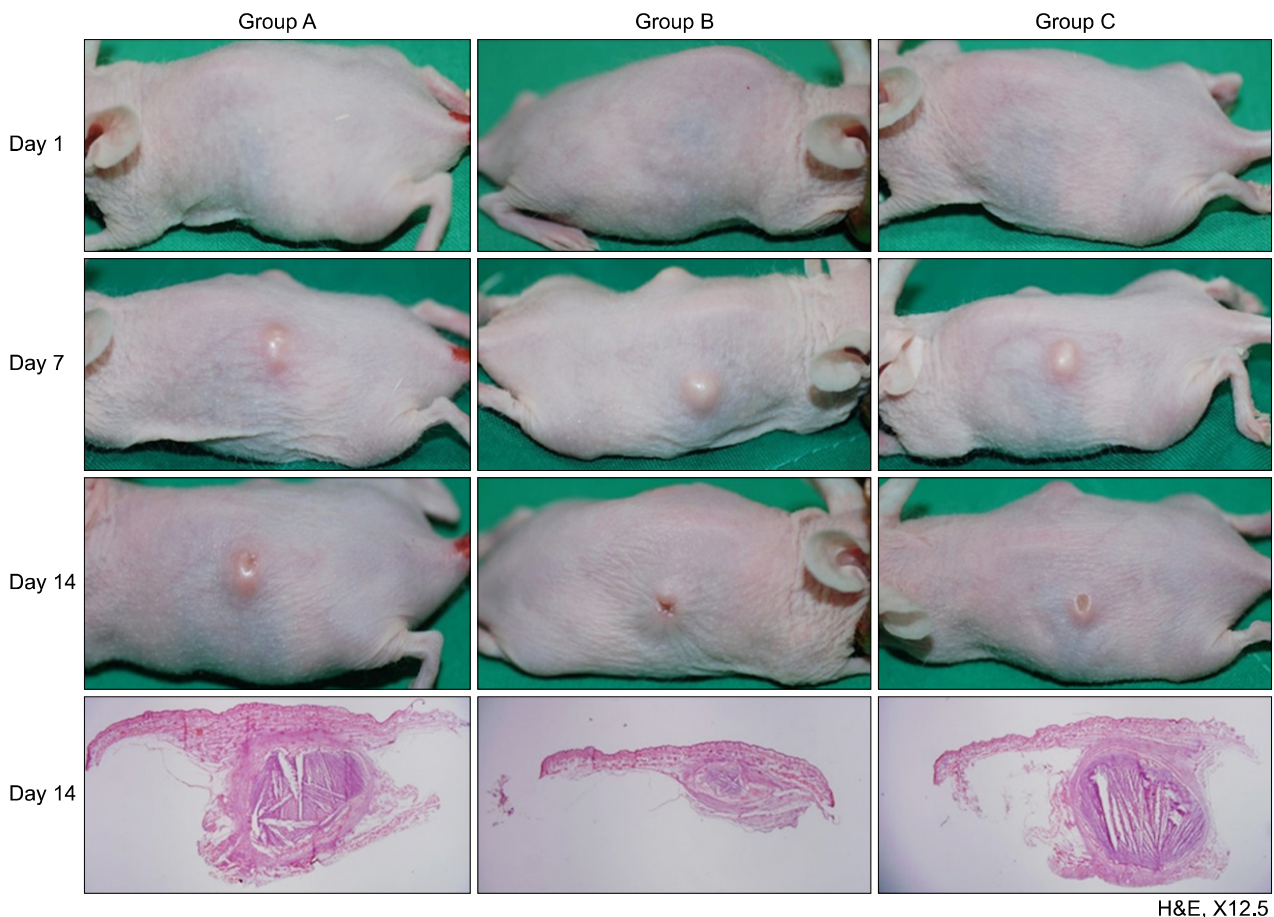
min), and lyophilized prior to injection. A *P. acnes* suspension was prepared with a concentration of 10^9 colony-forming units/ μ l. With a 30-gauge needle, the *P. acnes* suspension was injected intradermally in 20- μ l aliquots on both sides of the mouse back. Four mice in group A were injected with *P. acnes* and were not treated with red or infrared LED. Three mice in group B were treated with red LED after *P. acnes* injection, and three mice in group C were treated with infrared LED after *P. acnes* injection. The animal study protocol was approved by the Ethics Committee for animal studies at Kyoungpook National University, Korea (KNU 2014-0135).

Light-emitting diode irradiation of the *Propionibacterium acnes*-injected mice

After *P. acnes* injection, the mice were irradiated using a 28.9 J/cm² 650-nm red LED in group B and a 9.3 J/cm² 830-nm infrared LED in group C once daily for 2 weeks.

Evaluation of clinical changes

The degree of clinical inflammatory changes was eval-



H&E, X12.5

Fig. 1. Clinical inflammatory lesions in group B and group C improved greatly compared to those in group A.

uated using digital photography at baseline and at weeks 1 and 2 after *P. acnes* injection.

Histological examination and immunohistochemical stains

Tissue samples of mice were taken 2 weeks after *P. acnes* injection. Eight tissue samples were collected from group A, 6 tissue samples from group B, and 6 tissue samples from group C. Paraffin-embedded tissue sections of 3- μ m thickness were processed for light microscopy. Hematoxylin and eosin (H&E) and immunohistochemical staining was performed using standard techniques. The primary antibodies were as follows: integrin α 6 (diluted 1:150; Santa Cruz Biotechnology Inc., Dallas, TX, USA), neutrophils (diluted 1:80; Abcam, Cambridge, UK), interleukin (IL)-1 β (diluted 1:150; Abcam), matrix metalloproteinase (MMP)-2 (diluted 1:300; Abcam), and MMP-9 (diluted 1:250; Abcam). The histological changes in each group were evaluated semiquantitatively with a 3-point scale: mild (+), moderate (++), and prominent (+++), with an emphasis on changes in inflammation, epidermal/follicular wall thickness, and formation of cystic structures containing keratinized plugs (microcomedone-like cystic structures) in the dermis. The degree of immunohistochemical staining in each group was also evaluated semi-

quantitatively with a 3-point scale: mild (+), moderate (++), and dense (+++).

RESULTS

Clinical changes

Improvement in the inflammatory nodules of group B and group C was compared to that observed in group A (Fig. 1,

Table 1. Summary of the histological analysis and immunohistochemical profile results

Variable	Group A	Group B	Group C
Clinical inflammation	+++	+	++
Epidermal thickening	+	+	+
Microcomedone-like cysts	++	++	++
Epidermal proliferation:	++	++	++
Integrin α 6			
Inflammatory cells:	+++	+	++
Neutrophils			
Inflammatory markers:	++	+	+
IL-1 β , MMP-2/9			

Group A: *P. acnes*-treated group, Group B: *P. acnes* and red LED-treated group, Group C: *P. acnes* and infrared LED-treated group. IL: interleukin, MMP: matrix metalloproteinase, +: mild, ++: moderate, +++: prominent or dense.

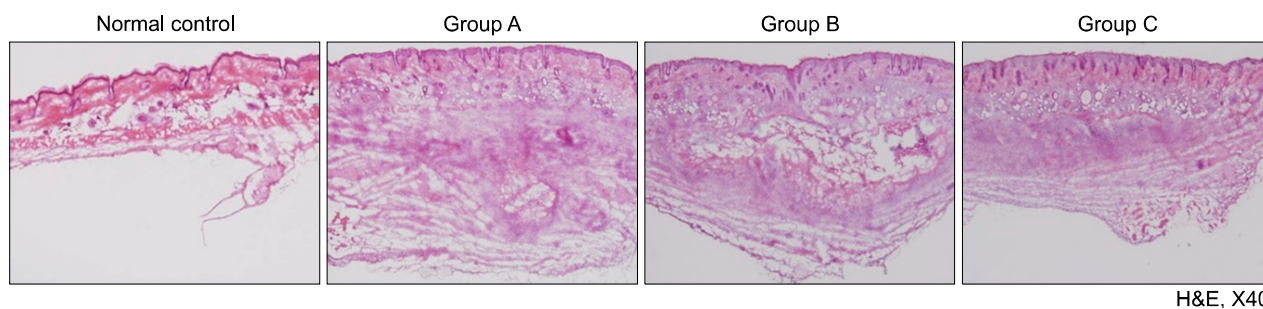


Fig. 2. Improvement in epidermal hyperplasia and microcomedone-like cysts in group B and group C was similar to that in group A.

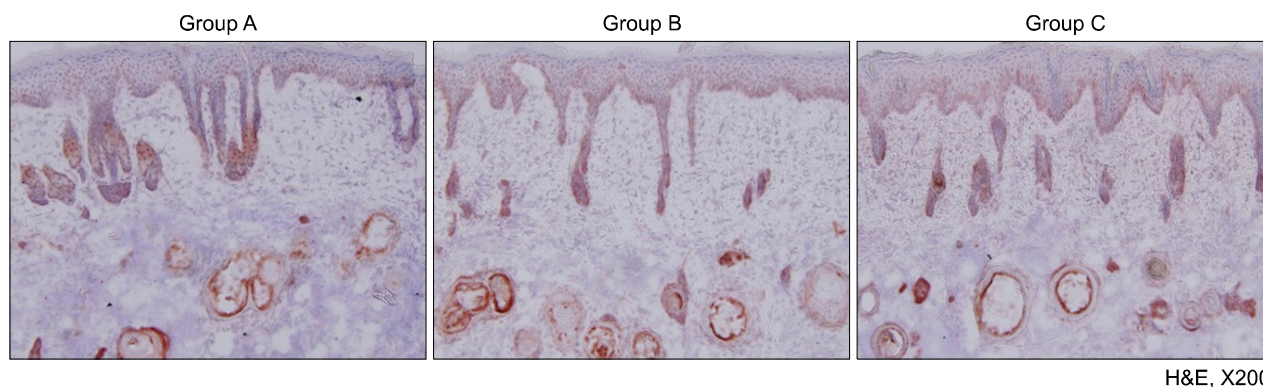


Fig. 3. Integrin α 6 expression in group B and group C was similar to that in group A.

Table 1). Group B showed more improvement in inflammatory nodules than group C, and group C showed more improvement in inflammatory nodules than group A.

Histopathological findings

Tissue samples obtained from the three groups (A, B, and C) were stained with H&E. Stains from the treatment groups were compared to those of the control group without *P. acnes* injection. Improvement in epidermal hyperplasia and thickening in group B and group C was similar to that observed in group A (Fig. 2, Table 1). The number and size of the microcomedone-like cysts in the upper dermis above the focus of inflammation in group B and group C decreased to an extent similar to that in group A (Fig. 2, Table 1).

Immunohistochemical stains

1) Epidermal proliferation marker: integrin $\alpha 6$

Improvement in the expression of integrin $\alpha 6$, the marker for epidermal proliferation, in the basal epidermis and microcomedone-like cyst wall in group B and group C was similar to that in group A (Fig. 3, Table 1).

2) Inflammatory cells: neutrophils

The infiltration of neutrophils into the dermis was decreased in group B and group C compared to that observed in group A (Fig. 4, Table 1). The infiltration of neutrophils in group B decreased to a greater extent than that in group C.

3) Inflammatory markers: interleukin-1 β

IL-1 β expression in the basal epidermis after treatment decreased in group B and group C compared to that in group A (Fig. 5, Table 1). IL-1 β expression decreased to a greater extent in group B than in group C.

4) Tissue remodeling markers: matrix metalloproteinase-2 and matrix metalloproteinase-9

MMP-2 expression in the dermis decreased to a greater extent in group B and group C than in group A (Fig. 6, Table 1). MMP-9 expression in the dermis decreased to a greater extent in group B and group C than in group A (Fig. 6, Table 1).

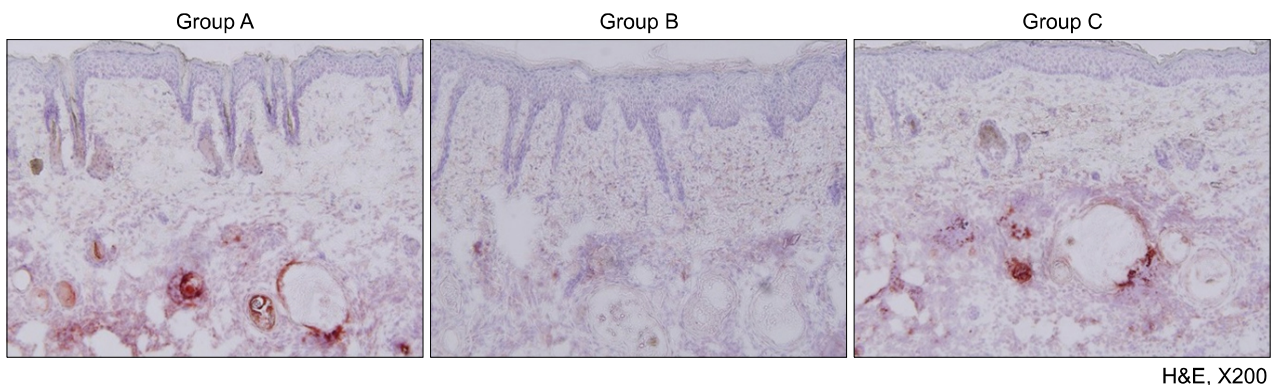


Fig. 4. Neutrophil infiltration decreased in group B and group C compared to that in group A.

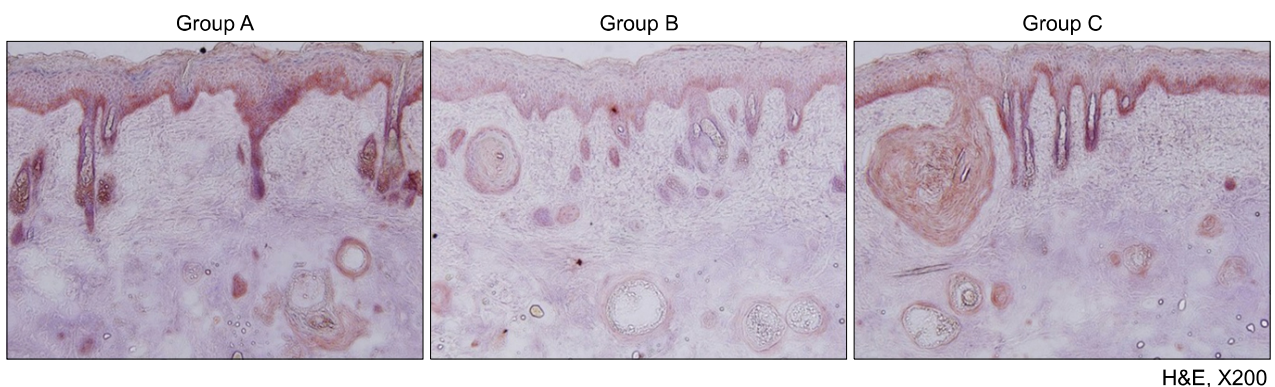
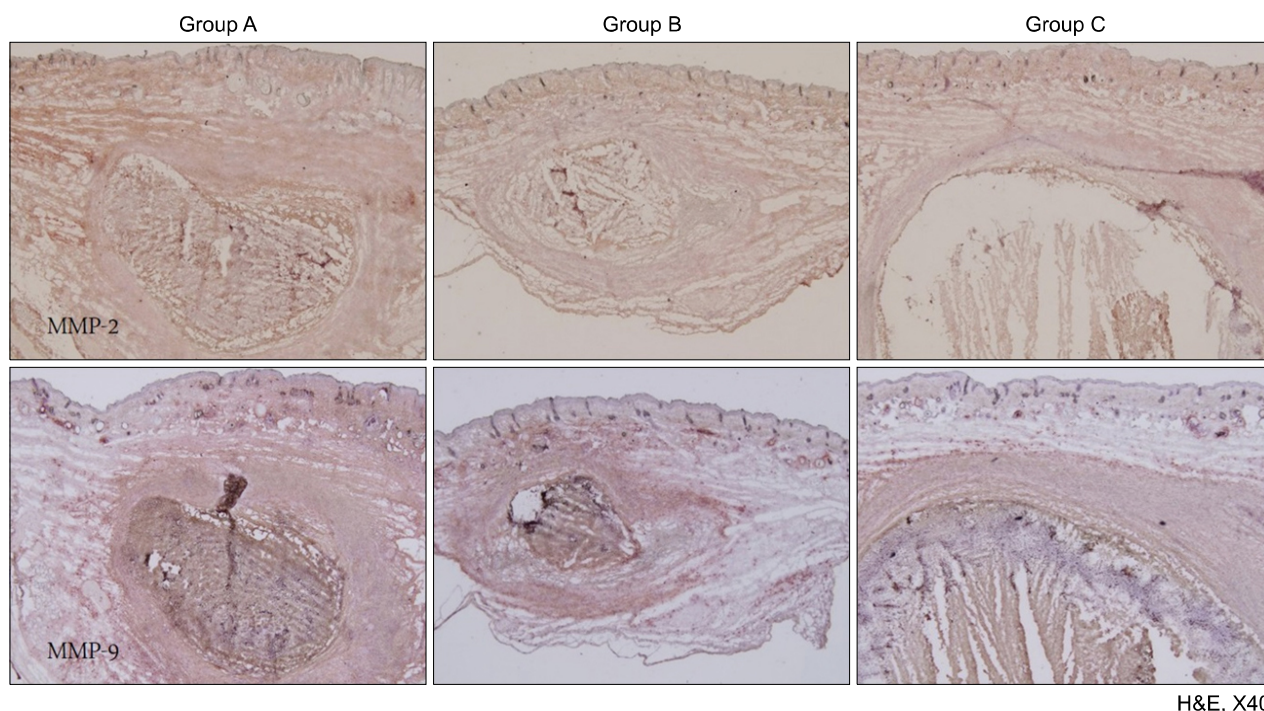


Fig. 5. Interleukin-1 β expression decreased in group B and group C compared to that in group A.



H&E, X40

Fig. 6. Matrix metalloproteinase (MMP)-2 and MMP-9 expression decreased greatly in group B and group C compared to that in group A.

DISCUSSION

Currently, several animal models can be used for acne research. Among them, rabbit ears and Rhino mice have been used frequently to determine the compound comedogenicity of acne lesions^{6,7}. However, the rabbit-ear and Rhino mice models have some limitations, including a lack of bacterial colonization and inflammation^{4,7}. Therefore, these models are not helpful for inflammatory acne research. In addition, the use of rabbits may be inconvenient for large-scale drug screening and vaccinations. As proven by our previous study, *P. acnes* injection into the HR-1 mouse induces acneiform inflammatory nodules with overlying epidermal hyperplasia and superficial secondary microcomedones. Therefore, the HR-1 mouse is suitable for inflammatory acne research.

In this study, HR-1 mice were used to determine the efficacy of red or infrared LED treatment of inflammatory acne. Laser/light-based devices are an alternative to conventional acne therapeutics in some patients who experience side effects or recurrence with conventional treatments. Treatment with ultraviolet (UV) A and UVB was found to have a marginal beneficial effect for acne⁸. Blue light is most effective in the photoactivation of the endogenous porphyrin component of *P. acnes*, because the 407~420-nm wavelength light has the strongest porphyrin photoexcitation coefficient. However, blue light

cannot access the deeper dermis⁹. On the contrary, red light penetrates deeper to the sebaceous glands and has anti-inflammatory properties, as apparent from its influence on cytokine release from macrophages^{10,11}. According to an action spectrum for the inactivation of *P. acnes*, the sensitivity of *P. acnes* is the highest with shorter wavelengths and decreases with increasing wavelength¹². A combination of blue and red LED application appears to have excellent potential in the treatment of mild-to-severe acne¹³. The infrared laser light, at such strengths as 1,450 nm and 1,540 nm, selectively produces an injury zone in the dermis, where the sebaceous glands are located, sufficient enough to inhibit high sebum production, leading to acne improvement⁹.

In this study, both red and infrared LED had anti-inflammatory effects on HR-1 mice with inflammatory nodules composed mainly of neutrophils and histiocytes. Red LED was much more effective than infrared LED. A decrease in the expression of inflammatory markers, including neutrophils, IL-1 β , MMP-2, and MMP-9, was shown in HR-1 mice after treatment with red or infrared LED. Their expressions after treatment with red LED decreased to a greater extent than those after treatment with infrared LED. However, the epidermal hyperplasia of the HR-1 mice after red or infrared LED treatment was similar to that after no LED treatment. The size and number of microcomedone-like cysts after red or infrared LED treatment

were similar to those after no LED treatment. In addition, the expression of integrin $\alpha 6$ after red or infrared LED treatment was similar to that after no LED treatment. Unfortunately, a limitation to this study is that these animal models do not fully represent inflammatory acne.

In conclusion, red or infrared LED has the potential to relieve inflammatory acne and decrease the expression of inflammatory biomarkers; red LED is much more effective than infrared LED. HR-1 mice with *P. acnes*-induced inflammation represent a good model for the investigation of inflammatory acne.

ACKNOWLEDGMENT

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2007017); Daegu-yeong Institute for Regional Program Evaluation.

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