ผลของกลาสไอโอโนเมอร์ซีเมนต์ชนิดใหม่ต่อเซลล์สร้างกระดูกมนุษย์

Effect of Novel Glass-ionomer Cement on Human Osteoblasts

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บทคัดย่อ

งานวิจัยนี้ได้ทำการศึกษาคุณสมบัติทางชีวภาพของกลาสไอโอโนเมอร์ซีเมนต์ชนิดใหม่ที่มีการเติมโปรตีนลูกผสม *Pmer*-TCTP ต่อเซลล์สร้างกระดูกมนุษย์ โดยใช้เทคนิค BrdU assay เพื่อตรวจการเพิ่มจำนวนของเซลล์ จากการทดลองพบว่าชิ้นงานที่เติมโปรตีน *Pmer*-TCTP ปริมาณ 0.2 -2 นาโนกรัมต่อชิ้นงานขนาดเส้นผ่านศูนย์กลาง 7 มิลลิเมตร หนา 1 มิลลิเมตร มีการเพิ่มจำนวนของเซลล์อย่างมีนัยสำคัญ (P < 0.05) โดยมีปริมาณเซลล์สร้างกระดูกมากที่สุดเมื่อเทียบกับชิ้นงานที่ไม่เติม *Pmer*-TCTP จากการศึกษาวิจัยนี้อาจทำให้เกิดการพัฒนาวัสดุชีวภาพทางทันตกรรมชนิดใหม่ซึ่งสามารถนำไปประยุกต์ใช้ในวิศวกรรมเนื้อเยื่อกระดูกในอนาคต

ABSTRACT

This research emphasized the biological effect of a novel glass ionomer cement supplemented with *Penaeus merguiensis*-translationally controlled tumor protein (*Pmer*-TCTP) on human osteoblasts (NHost cell). The cell proliferation was investigated by for the BrdU assay. It was found that the glass ionomer cement with added *Pmer*-TCTP at 0.2-2 ng per specimen (7 mm diameter and 1 mm thickness) can significantly promote NHost cell proliferation (P < 0.05) compared to the specimen without *Pmer*-TCTP. This study may lead to the development of a new dental biomaterial that may use for bone engineering in the future.

คำสำคัญ: โปรตีน *Pmer*-TCTP, ไคโตซาน, เซลล์สร้างกระดูกมนุษย์, กลาสไอโอโนเมอร์ซีเมนต์

Keywords: translationally controlled tumor protein (*Pmer*-TCTP), chitosan, human osteoblasts (NHost cell), glass-ionomer cements

INTRODUCTION

The bone therapeutic approaches include bone graft transplants, implants of various biomaterial and bone transplantation methods (Cancedda *et al.*, 2007). However, none has been proven to be fully satisfactory. Chitosan have been widely used for application in bone reconstruction as the material implants and play a critical role in tissue engineering. Chitosan is a biopolymer and has considerably been employed as a scaffold in orthopedic and other biomedical application (Jayakmar *et al.*, 2005) due to its biocompatibility, biodegradability, pore formation behavior, suitability for cell ingrowth.

Glass ionomer cements (GIC) have been used in various dental and medical application (Martin *et al.*, 2006), primarily due to their biocompatibility, antibacterial properties, ion leachability, and capacity to bond to bone, enamel, dentine and metals. GIC are products of an acid-base reaction between polyalkenoic acids, mainly poly (acrylic acid), and fluoro-aluminosilicate glass (Rakkiettiwong *et al.*, 2011).

Translationally controlled tumor protein (TCTP) is a highly conserved protein that is widely expressed in all eukaryotic organisms. It was used as an anti-apoptotic protein (Bangrak *et al.*, 2004). Previous study reported that TCTP can be protected HDPCs death form the cytotoxicity of resin glass ionomer cement and promote mineralization of pulp cells (Wanachottrakul *et al.*, 2011). Therefore, it is of interest to study the effect of TCTP with supplement in GIC. The hypothesis of this study is to improve biological property of novel GIC by adding TCTP. This novel GIC has been formulated with chitosan in order to prolong releasing of added protein. The objective of this study is to investigate the proliferative effect of this novel GIC supplemented with TCTP on human osteoblasts.

MATERIALS AND METHODS

1. Cell Culture

Normal human osteoblast cells (NHost cell) from human long bones were obtained form Cambrex (Lonza, USA). The cells were grown in 10 ml osteoblast growth medium (Alpha-modified Eagle's medium) at 37 °C in a humidified atmosphere containing 5% CO_2. The culture medium was replenished every 2-3 days. After reaching 90% confluence, cells were trypsinized using 0.05% trypsin with EDTA. NHost cells from passage 2-6 were used for the study.

2. Expression and purification of *Penaeus merguiensis*-translationally controlled tumor protein (*Pmer*-TCTP)

TCTP gene that was derived from *Penaeus merguiensis* was cloned and expression in bacterial Escherichia coli (*E.coli*) strain BL21. The *E.coli* strain BL21 harboring pGEX-*Pmer* TCTP was inoculated and induced. After induction, the bacteria cells were harvested by centrifugation and purified by using Glutathione Sepharose 4 Fast Flow (GE Healthcare Bio Science, Piscataway, NJ, USA) and thrombin was used for cleavage of GST-tagged protein. The molecular mass of purified TCTP protein is about 19.2 kDa.

3. Specimen preparation

GIC used in this study was a conventional material. The powder was composed of calcium fluoroaluminosilicate glass and poly acrylic acid. The novel GIC, called BIO-GIC, was prepared with chitosan and BSA. BIO-GIC powder was mixed with the liquid of conventional GIC. Disk specimens were prepared and mixed by using a stainless steel spatula. The specimen was then packed into split ring Teflon molds (7 mm diameter and 1 mm thickness) by using the mixing spatula. A polythene sheet and glass slide were then placed over the filled mold, after which firm hand pressure was applied.

4. Cell Proliferation ELISA, BrdU (colorimetric) assay

The Cell Proliferation ELISA is designed as a precise, fast and simple colorimetric alternative to quantitate cell proliferation based on the measurement of BrdU incorporation during DNA synthesis in proliferation cell. Thus, the Cell Proliferation ELISA can be used in many different in vitro cell systems when cell proliferation has to be determined. An important development has been the replacement of [3H]-thymidine by 5-bromo-2'-deoxyuridine (BrdU). This technique is based on the incorporation of the pyrimidine analogue BrdU instead of thymidine in to the DNA of proliferating cells. After its incorporation into DNA, BrdU is detected by the measurement of the absorbance of the samples in an ELISA reader at 450 nm (reference wavelength 690 nm).

5. Statistical analysis

The result data was analyzed for normality testing using Shapiro and Wilk method. The quantitative data performed in Cell Proliferation ELISA (BrdU) experiment was expressed as mean ± standard deviation. Statistical significance was evaluated using the one-way ANOVA and Tukey post hoc test. *P*-values less than 0.05 will be considered as statistically significant.

RESULTS AND DISCUSSION

1. Expression of TCTP form pGEX-*Pmer-TCTP*

TCTP gene that was derived from *Penaeus merguiensis* was cloned and expressed in bacterial Escherichia coli (*E.coli*) strain BL21 pGEX. The *Pmer*-TCTP had molecular weight at 48 kDa on 12% SDS-PAGE as shown in Figure 1.

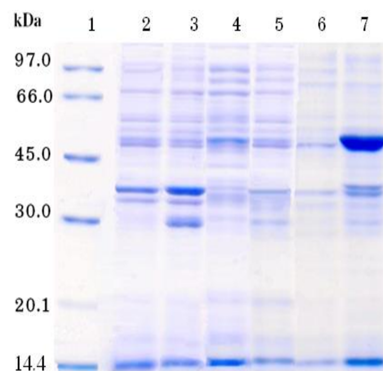

Figure 1 *Pmer*-TCTP in 12% SDS-PAGE (1) low molecular weight standard marker, (2) non induce pGEX insoluble, (3) induce pGEX insoluble, (4) non induce pGEX soluble, (5) induce pGEX soluble, (6) non induce pGEX-TCTP insoluble, (7) induce pGEX-TCTP insoluble (48kDa).

2. Cell Proliferation assay

The results of cell proliferation effect were reported by cell indexes, as show in Figure 2. All cell indexes of glass-ionomer cement groups were statistically less than the control group which revealed the toxicity of the specimens except the two groups of specimens with added TCTP at 0.2 and 2 ng per specimen had significantly higher cell indexes than the control and other groups (P<,0.05). This result supported that GIC supplemented with TCTP can promote growth of NHost cells.

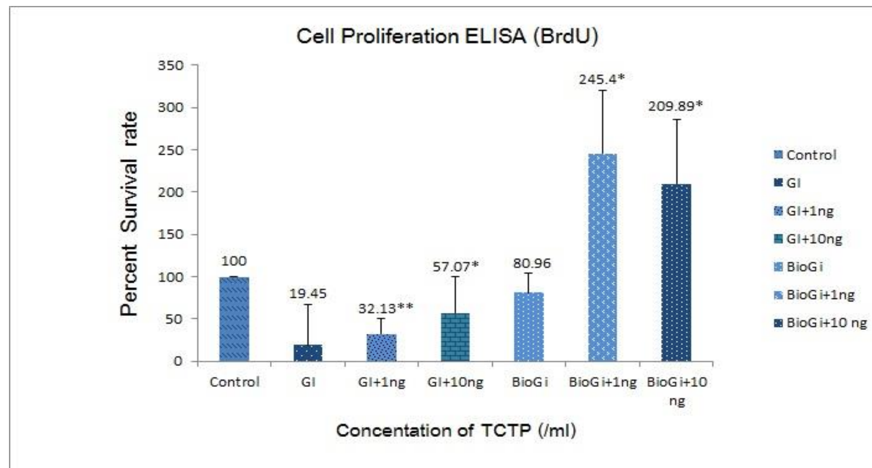

Figure 2 Human osteoblasts (NHost) cell proliferation by BrdU assay.

CONCLUSION

The novel GIC supplemented with translationally controlled tumor protein from banana prawn (*Penaeus merguiensis*) has an ability to promote NHost cell proliferation.

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