

Original Research Paper

Functional Evaluation of Polydioxanone Threads Coated with Polyethylene Glycol and Amino Acids for Medical Application

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Abstract: Polydioxanone (PDO) is a biodegradable polymer with multiple medical applications. Recently, various coatings have been applied to products made of biodegradable polymers, in order to improve functionality. Polyethylene Glycol (PEG) hydrogels have demonstrated remarkable potential in accelerating wound healing and amino acids have been reported to stimulate protein synthesis *in vivo* and *in vitro*. The purpose of this study was to investigate the biocompatibility and functionality of the PDO thread coated with a 1% (w/w) coating solution containing PEG and amino acids Glycine (Gly) and L-proline (L-proline). In particular, we evaluated whether this coating enhanced wound regeneration ability and promoted collagen synthesis. After coating PDO threads with PEG, Gly, and L-Pro alone or in combination, the cytotoxicity, antioxidant activity, and collagen production were examined in cultured human skin fibroblasts. All experiments were repeated three times. All coated threads exhibited low cytotoxicity and the threads coated with PEG or PEG and L-Pro showed higher antioxidant activity and greater collagen production in human fibroblasts than the uncoated threads. Our findings suggest that PDO threads coated with PEG and amino acids are biocompatible and offer several beneficial properties, including antioxidant microenvironment formation and wound healing stimulation, offering the potential for use in various medical applications.

Keywords: Antioxidant Activity, Biocompatibility, Collagen Production, Cytotoxicity, Polydioxanone

Introduction

Biodegradable polymers are extensively used in medical applications for manufacturing surgical materials, sutures, and drug delivery systems (Prajapati *et al.*, 2019). Polydioxanone (PDO) is a typical biodegradable polymer that was commercialized in the 1990s. PDO has more flexibility than polyglycolic acid and is used for the preparation of monofilament threads for surgical sutures, as well as for wrinkle improvement and skin lifting in cosmetic surgery (Goonoo *et al.*, 2015).

It is important that wound closure using medical thread be performed stably. It has been common practice to coat multifilament sutures to improve their physical properties, such as smoothening the surface (Regula and Yag-Howard, 2015; Martins *et al.*, 2020; Lekic and Dodds, 2022; Wu *et al.*, 2022).

Recently, various coatings have been applied to products made of biodegradable polymers to add

functionality. Coating thread surfaces with silver nanoparticles and chlorhexidine improves anti-inflammatory properties and better functional properties and convenience of use have been achieved by coating polymer threads with hyaluronic acid and chitosan (Liu *et al.*, 2021; Wu *et al.*, 2017; Mohammadi *et al.*, 2020; Zhou *et al.*, 2021; Ademuyiwa *et al.*, 2022).

The main purpose of medical threads is to enhance the wound suturing effect. In the cosmetic surgery field, there is a high demand for functional threads with collagen regeneration properties to improve wrinkles and make the skin clear (Chattopadhyay and Raines, 2014; Aitzetmueller *et al.*, 2019; Lekic and Dodds, 2022).

Among synthetic polymers, Polyethylene Glycol (PEG), a polyether-based amphipathic compound, has found many applications in tissue engineering owing to its ability to blend with different polymers. PEG has excellent biocompatibility, low immunogenicity, and low toxicity and it is widely used in the production of pharmaceuticals,

cosmetics, and medical devices. Specifically, medical devices may be coated with PEG to improve their usability and biocompatibility. PEG hydrogels show great potential in accelerating wound healing (D'souza and Shegokar, 2016; Kalai Selvan *et al.*, 2020; Liu *et al.*, 2021).

Amino acids are the building blocks of proteins and they can be involved in protein synthesis. Collagen is present in a significant amount in our body. Glycine (Gly) and L-Proline (L-Pro), the basic building blocks of proteins, account for 57% of the total amino acid content of collagen, which accounts for one-third of the total protein content of animals. Gly and L-Pro are used to synthesize polypeptide chains to form collagen, the body's most abundant protein, and are essential for the synthesis of elastin (Li and Wu, 2018; Scarano *et al.*, 2021).

In this study, we sought to evaluate PDO threads coated with PEG, Gly, and L-Pro (alone and in combination, as medical threads for potential clinical use in promoting regeneration and collagen synthesis at wound sites. The cytotoxicity of coated medical PDO threads was evaluated to confirm biocompatibility and wound regeneration and collagen synthesis abilities of PDO threads were examined.

Materials and Methods

Materials

The PDO thread used in the experiment was size 2 as per United States Pharmacopeia and was purchased from PINE BM Co., Ltd. (Daejeon, South Korea). PEG and Gly used in the experiment were purchased from WonPoong Pharm. Co., Ltd. (Hwaseong, South Korea) and L-Pro were purchased from Sigma-Aldrich (Merck Korea, Seoul, South Korea).

Preparation of Coating PDO Threads

For the surface coating of PDO medical thread, 1% (w/w) coating solution was prepared for each condition. PEG, Gly, and L-Pro were dissolved in 10 mL of distilled water to make a coating solution; PDO threads were segmented into 90 cm-long pieces and submerged in a coating solution. PDO threads were coated by dipping using a feeding roller at a speed of 8 cm/min and after dipping, it was dried in a drying oven at 40° for 6 h (FC-PO-250, LAB House, Seoul, South Korea). Table 1 shows the composition of the prepared coating solutions.

Table 1: Composition of coating solutions

Sample type	Coating type
A	Gly
B	Pro
C	PEG
D	Gly + PEG
E	Pro + PEG
F	Gly + Pro
G	Gly + Pro + PEG

Preparation of Samples Extracts of Coated PDO Thread

To determine the biocompatibility of the coated threads, cell viability was evaluated using the cytotoxicity test, and an indirect method of obtaining material extract was used to measure the amount of collagen produced by applying the coating thread to human skin fibroblasts (ANSI/AAMI/ISO, 2009; 2012). The extraction ratio was calculated as 0.2 g/mL, based on the ratio of surface area to extraction volume, following established guidelines (Biological evaluation of medical devices, part 12: Sample preparation and reference materials). Subsequent to cutting and sterilization, the sample underwent immersion in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Carlsbad, CA, USA) supplemented with 10% Fetal Bovine Serum (FBS) at a controlled temperature of 37±1°C for 24 h.

Antioxidant Activity Measurement

The antioxidant activity of the coated thread samples was assessed using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay kit purchased from Biomax Co., Ltd., following the procedural guidelines provided by the manufacturer (Biomax, Seoul, South Korea). Extracts from threads coated under various conditions, along with Trolox standards and the DPPH working solution, were individually dispensed into separate wells of a 96-well plate and thoroughly mixed by vortexing (total volume: 200 µL per well). The reaction was conducted under light-protected conditions at 25°C for 30 min, followed by absorbance measurement at 517 nm using a microplate reader (FlexStation® 3 multi-mode microplate reader, molecular devices, San Jose, CA, USA). Antioxidant activity was subsequently calculated:

$$\text{Antioxidant activity (\%)} = \frac{Abs_c - Abs_s}{Abs_c} \times 100$$

Abs_c = Absorbance of control

Abs_s = Absorbance of sample

Cell Culture

Human skin fibroblasts (CCD-986sk, Korean cell line bank, Seoul, South Korea), obtained from the Korea cell line bank, were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 95% air and 5% CO₂. Cells were seeded at a density of 1×10⁴ cells per well in 96-well plates. The study protocol received approval from the research ethics review committee (No. DKU NON2023-001).

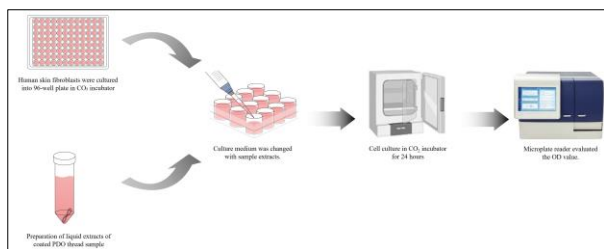


Fig. 1: Graphical scheme of the cell culture experiment

In vitro Cell Viability Assessment

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit (Abcam, Boston, MA, USA). Human skin fibroblasts were seeded into individual wells of 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h. Subsequently, the MTT assay was conducted following 24 h of exposure to the test substance, adhering strictly to the manufacturer's instructions (Fig. 1). Absorbance was measured at 590 nm using a microplate reader (FlexStation® 3 Multi-Mode microplate reader, molecular devices). Cytotoxicity (%) was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{100 \times (\text{Background Control} - \text{Sample})}{\text{Background Control}}$$

Background Control: Absorbance value of medium containing FBS

Collagen Quantification via Enzyme-Linked Immunosorbent Assay

Human skin fibroblasts were cultured overnight in 96-well plates, following which the control medium was substituted with the same medium supplemented with the sample extract. The cells were further grown with the extracts of thread samples for 24 h. After culturing, the supernatants were collected from each well and analyzed for the amount of newly synthesized type I procollagen. We used a procollagen type I C-Peptide Enzyme Immunoassay (EIA) kit from TakaRa Biomedical Inc., (Seoul, South Korea), according to the manufacturer's instructions. Subsequently, the absorbance of the solution was measured at 450 nm using a microplate reader (FlexStation® 3 multi-mode microplate reader, molecular devices).

Statistical Analysis

The results obtained both pre- and post-coating were analyzed by one-way analysis of variance (ANOVA) and

independent samples t-test using SPSS software (Version 18.0, SPSS Inc., Chicago, IL, USA). Data are presented as mean \pm standard deviation, with statistical significance considered at $p < 0.05$.

Results

Antioxidant Activity of the Threads

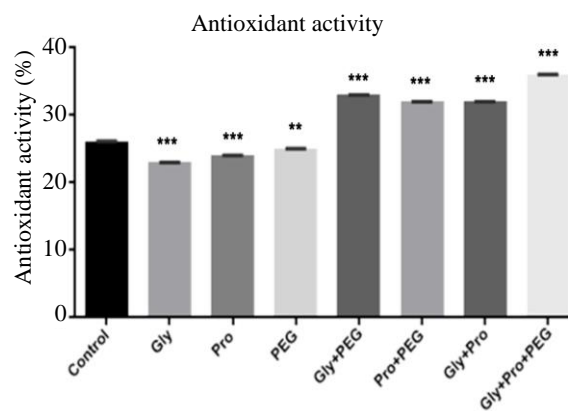
Figure 2(A), the threads coated with both PEG and one amino acid showed higher antioxidant activity than those coated either with PEG or an amino acid alone. Extracts of threads coated with PEG and both L-Pro and Gly showed the highest antioxidant activity.

Effect of Coated Threads on Cell Viability

To evaluate the potential biomedical applications of the coated threads, human skin fibroblast cell lines were cultured for 24 h with thread extracts to assess cytocompatibility. Figure 2(B), cell viability was not compromised following culture with extracts of uncoated and coated threads, as evidenced by the results of the MTT assay. According to the ISO 10993 standard, if the viability of cells affected by a substance is 70% or higher, the substance is considered to lack a direct cytotoxic action. Given that the cell viability in cultures with extracts of all types of coated threads ranged between 100 and 155%, we concluded that the coated threads did not show cytotoxicity and could be safely used in medical applications.

Collagen Production Quantification Via Enzyme-Linked Immunosorbent Assay

The collagen production by fibroblasts in the presence of extracts of threads coated with both Gly and L-Pro was the lowest, whereas it was the highest when the cells were cultured with extracts of threads coated with PEG alone or L-Pro and PEG together (Fig. 2(C)).



(a)

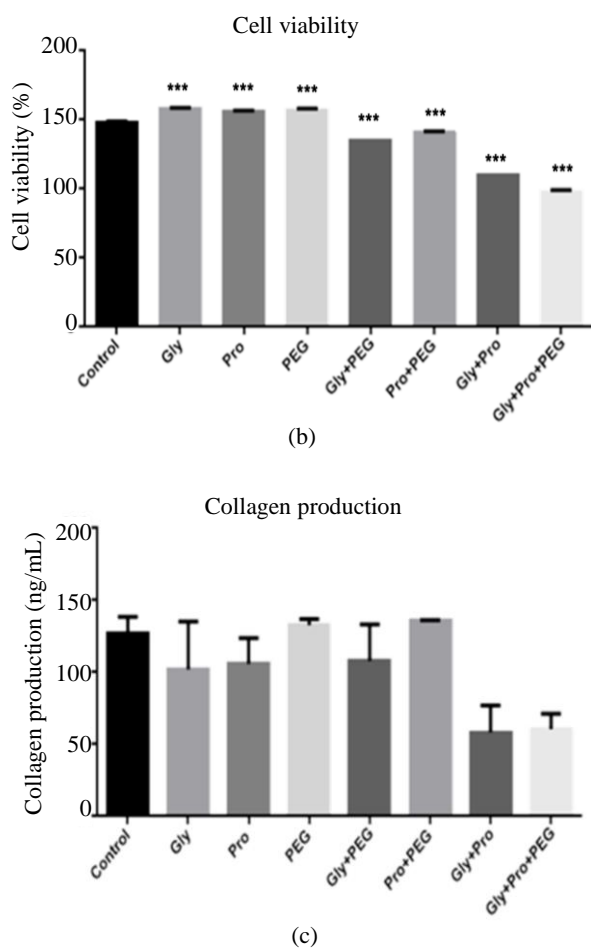


Fig. 2: Functional evaluation of the coated threads using cultures of human skin fibroblasts incubated with thread extracts. (A) Antioxidant activity; (B) cell viability (%); (C) collagen production (ng/mL). Values are presented as the mean \pm standard deviation ($n = 3$), and the level of significance is expressed as follows: ** $p < 0.01$ and *** $p < 0.001$ compared to control values

Discussion

Doctors choose medical threads with different diameters and strengths for surgeries and treatments, depending on the wound site and purpose (Açan *et al.*, 2018; Choi *et al.*, 2020; Bezrouk *et al.*, 2020). These medical threads are used not only for surgical operations for suturing and ligation but also in the aesthetic field to induce collagen regeneration, improve wrinkles, and regenerate skin tissue (Hussey and Bagg, 2011; Aitzetmueller *et al.*, 2019).

In this study, we sought to confirm the biocompatibility and functionality of biodegradable polymeric PDO threads coated with PEG and/or the amino acids L-Pro and Gly through experiments on

human skin fibroblasts. The biocompatibility and safety of materials for biomedical devices is a fundamental requirement. In this study, the biocompatibility of threads before and after coating was assessed using the MTT assay, a well-established method for detecting potential cytotoxicity. The extracts of coated threads were tested according to the ISO 10993-5 cytotoxicity guidelines and extracts of all preparations tested were associated with cell viability values greater than 100%. Thus, the threads had a neutral or a mild positive effect on cell growth. Because the cell viability did not drop below 70%, which is an established cytotoxicity threshold, we concluded that the coated PDO threads were not cytotoxic (ANSI/AAMI/ISO, 2009; 2012; Martins *et al.*, 2020).

The DPPH and collagen production assays further affirmed the functionality of the coated threads. The results of the DPPH assay showed that the threads coated with PEG and both Gly and L-Pro had higher antioxidant activity than the uncoated threads. Medical threads are used for stitching wounds in injured tissues. Wounds often exhibit increased levels of reactive oxygen species, crucial for triggering the proliferation and differentiation of cells as well as the synthesis of extracellular matrix components. Increased reactive oxygen species levels contribute to oxidative stress, fostering pro-inflammatory conditions that prolong wound healing. Therefore, high antioxidant activity may promote wound healing while minimizing foreign body reactions because it can decrease the levels of reactive oxygen species (André-Lévigne *et al.*, 2017; Dunnill *et al.*, 2017; Yao *et al.*, 2019; Zhu *et al.*, 2021).

We examined collagen production by fibroblasts and found that it was higher when cells were incubated with extracts of threads coated with PEG alone or a combination of PEG and L-Pro than when the cells were treated with uncoated threads. Collagen engages in the process of tissue regeneration and repair by interacting with fibroblasts. Higher collagen production by fibroblasts exposed to extracts of coated threads indicates that the clinical application of these threads may positively affect regeneration and repair (Chattopadhyay and Raines, 2014; Mathew-Steiner *et al.*, 2021; Sharma *et al.*, 2022).

Given that we studied the properties of coated PDO threads *in vitro* using human skin fibroblasts, our results may not precisely predict the reaction to these materials in the body.

However, the significance of our experiments is in the demonstration of the possibility that coated PDO threads could promote wound healing by increasing cell survival, forming an antioxidant environment, and increasing collagen production in the tissue. Thus, our data could serve as the foundation for future studies in animal models and subsequent clinical trials aimed at exploring the use of coated PDO threads in various medical applications.

Conclusion

In conclusion, we investigated a range of biological effects of biodegradable medical PDO threads coated with PEG and/or two types of amino acids (Gly and L-Pro) on cultured human fibroblasts. We observed that all coated threads tested had low cytotoxicity, among which the threads coated with PEG or PEG and L-Pro had higher antioxidant activity and promoted greater collagen production than uncoated threads. Our findings confirmed that PDO threads coated with PEG and amino acids (Gly and L-Pro) are helpful in wound healing and collagen synthesis, suggesting promising potential for their clinical use as medical threads.

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Author's Contributions

Eun Ji Park and Tae Su Jang: Made substantial contributions to the conception or design of the study.

Jae Kyung Kim: Made substantial contributions to the acquisition and analysis of data.

Ethics

This study was conducted after receiving approval from the Research Ethics Review Committee (approval no. DKU NON2023-001).

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