Improvement of Osseointegration by Amine modification on PEEK by plasma technology

Takuya Furuichi[1], Takahiro Kitahara[1], Masato Ikuta[1], Masayuki Bun[1], Hiromasa Hirai[1], Seiji Okada[1], Takashi Kaito[1 2]
[1]Osaka University, Osaka, Japan, [2]Osaka Rosai Hospital, Osaka, Japan
Email: u444821g@ecs.osaka-u.ac.jp

Disclosures: Takuya Furuichi (N), Takahiro Kitahara (N), Masato Ikuta (N), Masayuki Bun (N), Hiromasa Hirai (N), Seiji Okada (N), Takashi Kaito (N)

INTRODUCTION: PEEK (PolyEtherEtherKetone) possesses favorable properties such as biocompatibility, heat, chemical, and wear resistance, but one of the primary drawbacks of PEEK materials is that they do not naturally bond well with bone tissue (low osseointegration capacity). The addition of osteointegration capacity to PEEK enhances the advantages of PEEK as a biomaterial. We have reported that Amine(-NH_3) modification of artificial bone (calcium phosphates) surfaces using plasma technology enhances cell adhesion, osteogenic differentiation, and bone formation. The purpose of this study is to verify the effect of amine modification of PEEK on osteointegration and to elucidate the mechanism.

METHODS: Plasma discharge was applied with gas mixture containing N_2, CH_4, and He onto Cylindrical PEEK (5mm in diameter and height in 2mm) to perform Amine modification (Kodama, et al. Sci Rep 2021). The elemental composition ratio of the Amine coated PEEK surface was evaluated using X-ray photoelectron spectroscopy (XPS). Additionally, the wettability was assessed by measuring the water contact angle. For in vitro experiments, MC3T3E-1 osteoblast cell line was utilized. Cell cultures were conducted on both Amine-coated PEEK and untreated PEEK surfaces. ALP activity assessment was performed on days 3 and 7. For a more detailed examination of gene profiles, mouse mesenchymal stem cells (MSCs) were cultured on circular PEEK discs (32 mm in diameter) coated with Amine and Untreated PEEK discs for 1 and 5 days. Gene expression changes were examined through RNA sequencing and qPCR methods. Furthermore, signal changes related to osteogenic differentiation were validated using Western blot analysis. Cell adhesion evaluation involved culturing cells on PEEK surfaces for 2 hours, followed by observing cell morphology using Scanning Electron Microscope. Additionally, Non-adherent cells were removed using a centrifuge, and the remaining cell count was quantified by Image J software. In vivo experiments, a 3 mm diameter bone hole was created on the lateral side of the femoral condyle in 10-week-old SD rats. Amine coated and untreated PEEK discs (3 mm in diameter, 4 mm in height) were implanted on the left and right femoral condyles, respectively. The bone formation around the PEEK implants was histologically evaluated at postoperative 6 weeks. Statistical comparisons were made using the unpaired Student's t-test, and data were presented as mean ± standard deviation. A p-value of <0.05 was considered statistically significant. All animal experiments conducted in this study have received approval from our Animal Experiment Facility.

RESULTS: XPS indicated a significant deposition of nitrogen atoms on Amine coated PEEK. Taking into consideration our prior research findings, this suggested the deposition of Amine groups. The contact angle was decreased on Amine-modified PEEK, indicating an increase in hydrophilicity ($n=6$, $p<0.0001$). Culturing MC3TC-E1 cells on Amine coated PEEK, a significant increase in ALP activity was observed on day 7 ($n=4$, $p<0.05$). In RNA sequencing, Gene ontology (GO) and pathway analyses for the upregulated genes on day 5 indicated enrichment of terms such as ossification, ECM-receptor interaction, and Focal adhesion. Notably, the gene for the bone formation protein BMP4 was among the genes consistently upregulated from day 1 to day 5. Real time PCR analysis showed cells cultured on Amine coated PEEK exhibited elevated gene expression of Runx2, Osterix, Osteocalcin, and BMP4, as well as genes related to integrins (α1, α2, α4, β1). Western blot analysis confirmed the elevation of the MAPKs pathway following Focal adhesion kinase (FAK), and BMP/Smad signaling. In terms of cell adhesion evaluation, cells cultured on Amine-coated PEEK exhibited extended dendritic processes and a significantly increased number of residual cells after centrifugation. These findings suggest a strong cell-PEEK adhesive interaction. In vivo study, as the images below shows (Fig. A, B), bone formation without intervening soft tissue (osseointegration) was significantly more prevalent around Amine coated PEEK (Fig. C, $n=5$ in each group, $p<0.01$).

DISCUSSION: We have elucidated that Amine modification of PEEK surfaces using plasma technology promotes cell adhesion and osteoblast differentiation, consequently enhancing Osseointegration around PEEK. The increase in adhesion strength between the material and cells has been previously reported to lead to the phosphorylation of FAK and subsequent activation of MAPKs, thus promoting osteoblast differentiation. In the case of Amine-coated PEEK, not only was the phosphorylation of FAK enhanced, but also an increase in BMP4-induced SMAD signaling was observed. Although many aspects of this mechanism remain to be fully understood, it is considered that the positive charge carried by Amines attracts adhesive proteins to the PEEK surface, leading to enhanced cell adhesion and some form of osteogenic promotion. Through this technology, there is a high potential for the development of novel intervertebral cages that can maintain the mechanical properties inherent to conventional PEEK while promoting bone formation.

SIGNIFICANCE/CLINICAL RELEVANCE: The clinical significance of Amine coated PEEK lies in its capacity to stimulate bone differentiation, offering potential benefits for promoting bone growth and facilitating fusion.

IMAGES AND TABLES: