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Immobilization of Bone Morphogenic Protein-2 by Cold Atmospheric Plasma on PCL/CMC Scaffold for Osteo-Differentiation Application

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ABSTRACT

Introduction: The increasing demand for bone regeneration has created a need for functional substrates. An osteoconductive scaffold can direct stem cells to undergo osteo-differentiation without the use of growth factors. Physical surface modification using cold atmospheric plasma (CAP) is a novel approach for protein immobilization. This study aimed to utilize CAP for immobilizing bone morphogenic protein (BMP-2) on the surface of electrospun polycaprolactone (PCL) and carboxymethyl chitosan (CMC) (PCL/CMC) scaffolds.

Materials and Methods: The presence of BMP-2 on the electrospun PCL/CMC was confirmed by scanning electron microscopy (SEM). The biocompatibility and metabolic activity of the treated scaffold on human mesenchymal stem cells (hMSCs) were assessed using the MTT assay at 14 days. Osteo-differentiation of hMSCs was evaluated through gene expression of *RUNX2*, *SOX9*, *ALP*, and *Osteonectin*, along with protein expression of OSTEONECTIN, using PCR and immunocytochemistry (ICC), respectively.

Results and Discussion: CAP treatment successfully created functional groups capable of immobilizing BMP-2 on the PCL/CMC scaffold, as shown in SEM images. The scaffold exhibited no cytotoxic effects on hMSCs and supported cellular proliferation over 14 days ($p < 0.001$). PCR results indicated the expression of osteo-differentiation-related genes (*RUNX2*, ALP, and *Osteonectin*), while no band was observed for the *SOX9* gene. Additionally, OSTEONECTIN protein expression was detected in PCL/CMC/BMP-2 and CAP-treated PCL/CMC scaffolds.

Conclusion: The expression of both genes and proteins confirmed the osteoconductive potential of the designed scaffold. Ultimately, the CAP technique, combined with an appropriate scaffold structure and components, can induce osteo-differentiation without external supplemental media.

Keywords: Cold atmospheric plasma, Osteogenic differentiation, Protein immobilization, Tissue engineering

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