CURCUMIN LOADED P3HB-MCNTS ELECTROSPUN SCAFFOLDS – IN VITRO AND IN VIVO STUDY

MOEIN ZAREI*, MIROSLAWA EL FRAY*

DEPARTMENT OF POLYMER AND BIOMATERIALS SCIENCE, FACULTY OF CHEMICAL TECHNOLOGY AND ENGINEERING, WEST POMERANIAN UNIVERSITY OF TECHNOLOGY IN SZCZECIN, AL. PIASTÓW 45, 71-311 SZCZECIN, POLAND
*E-MAIL: ZM47832@ZUT.EDU.PL, MIRFRAY@ZUT.EDU.PL

[ENGINEERING OF BIOMATERIALS 163 (2021) 20]

Introduction

Electrospinning has recently attracted attention to create different nanofibrous structures emulating morphology of the extracellular matrix (ECM)[1]. Bacteria-synthesized biopolymers, such as poly(3-hydroxy butyrate) (P3HB), have triggered special attention in the development of various medical devices, soft and hard tissue engineering scaffolds and drug delivery systems[2]. However, P3HB electrospun scaffolds are not free from problems, as their lack of mechanical strength is a significant limitation for hard tissue engineering applications [1]. Therefore, different additives, such as functionalized multiwalled carbon nanotubes (MCNTs), can be introduced to improve not only mechanical properties, but also the biological response [1,3]. Another candidate with good anti-inflammatory and anti-oxidant properties is curcumin (CUR), which has been selected for reducing inflammation in different studies [4]. CUR has also been studied in combination with different polymers to produce electrospun scaffolds[5]. With this in mind, we combined P3HB-MCNTs for the first time with CUR to reduce the possibility of inflammation of electrospun scaffolds in their use as potential structures for bone tissue engineering applications.

Materials and Methods

P3HB powder was dissolved in chloroform/ dimethylformamide (7/3 v/v), MCNTs (0.5%wt) and CUR (10 and 20%wt) were added to the solution and loaded into the syringe for electrospinning device. The scaffolds morphology was evaluated using scanning electron microscopy (SEM) after 4 weeks immersing into the SBF (bioactivity) and PBS solution (biodegradability). Mesenchymal stem cells (MSCs) were cultured for 10 days (*in vitro* biocompatibility) and subcutaneous implantation of the scaffolds (*in vivo* biocompatibility) has been performed for 8 weeks in rat animal model.

Results and Discussion

The appearance of hydroxyapatite (HA) crystals on the surface of the fibers indicated the bioactivity of the scaffolds[6] (FIG. 1). The amount of sedimentary HA on the scaffolds with 20% of CUR was the highest indicating high bioactivity.

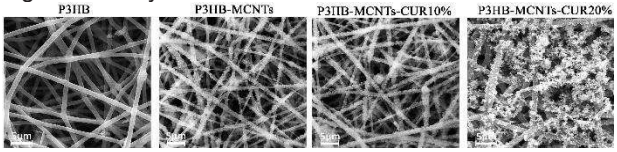

FIG. 1. SEM images after 4 weeks immersing in SBF.

FIG. 2 shows SEM pictures of the cultured cells attached onto the scaffolds after ten days (FIG. 2A) and the results from MTT assay at the first, fifth, and tenth day of seeding the MSCs cells onto the scaffolds (FIG. 2B). An increase in cell viability has been noticed for scaffolds containing 10wt% CUR, and significantly higher cell viability was observed for scaffolds loaded with 20% CUR.

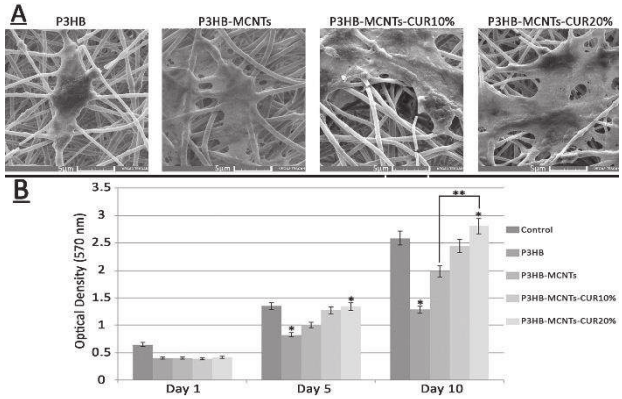

FIG. 2. SEM images of cell attachment onto the surface of the fibers (A) and MTT results (B)

The addition of 20% CUR increased the biodegradation rate to about 35% of mass loss after 4 weeks (FIG. 3). SEM micrographs of the scaffolds structure clearly indicate changes on the surface of the fibers.

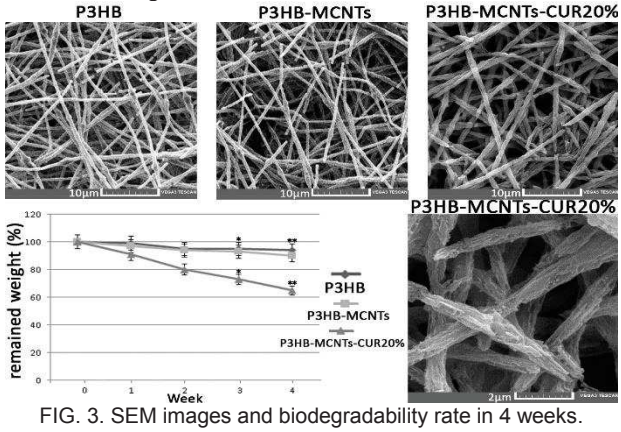

FIG. 3. SEM images and biodegradability rate in 4 weeks.

Scaffolds loaded with 20% of CUR were still present after 8 weeks of implantation (FIG. 4). Notably, there was less acute inflammation due to the presence of CUR as well as higher resorption of scaffolds. Some vessel formation around the scaffolds can also be seen.

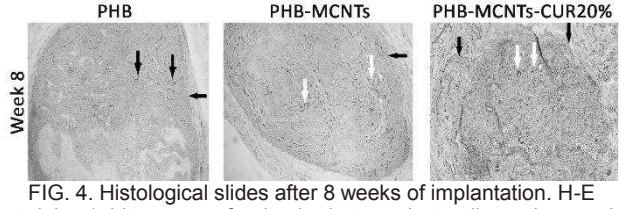

FIG. 4. Histological slides after 8 weeks of implantation. H-E staining (white arrows: foreign body type giant cell reactions and blue arrows: vessel formation around the scaffolds).

Conclusions

Composite scaffolds containing MCNTs and CUR showed accelerated hydrolytic degradation in PBS and enhanced hydroxyapatite precipitation from SBF as compared to neat P3HB. Moreover, CUR strongly reduced inflammatory reaction after 8 weeks of *in vivo* implantation of the scaffolds. Overall, our findings clearly indicate that electrospun scaffolds made of P3HB-MCNTs-CUR20% can be promising structures for tissue engineering applications.

References

[1] M. Zarei and S. Karbasi, J. Porous Mater. 25, 259 (2018).
[2] G. Q. Chen and Q. Wu, Biomaterials 26, 6565 (2005).
[3] P. A. Tran, L. Zhang, and T. J. Webster, Adv. Drug Deliv. Rev. 61, 1097 (2009).
[4] M. Mardani, A. Sadeghzadeh, N. Tanideh, A. Andisheh Tadbir, F. Lavaee, M. Zarei, and J. Moayedi, Iran. J. Basic Med. Sci. 23, 1618 (2020).
[5] A. Shababdoust, M. Ehsani, P. Shokrollahi, and M. Zandi, Prog. Biomater. (2018).
[6] T. Hiruta, T. Yabutsuka, S. Watanabe, K. Fukushima, S. Takai, and T. Yao, in *Key Eng. Mater.* (2017), pp. 69–74.