

Antibacterial membrane for bone regeneration sonocoated with nanoparticles - characterization and evaluation of cellular behavior in vitro

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Introduction

Insufficient bone volume to support teeth in the jaw is one of the most serious problems in dentistry [1]. Guided tissue regeneration (GTR) and guided bone regeneration (GBR) surgical procedures with separative membranes are often applied to restore missing bone [2,3] (Fig.1). In this work, we combined biocidal ZnO:Ag nanoparticles (nZnO:Ag) and bone growth stimulating nanohydroxyapatite (nHA) in a form of ultrasonically deposited coating on fibrous PDLLA/PLGA membranes (patented structure [4,5]). The aim was to create the structure able to minimize the microbial invasion during GTR/GBR procedures.

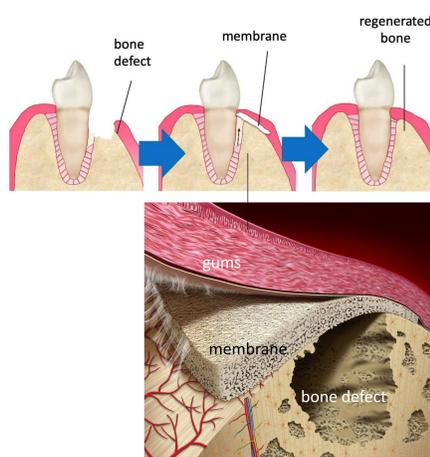


Fig.1 The principle of GTR procedure with biodegradable membrane. Source: Elgali et al. Guided bone regeneration: materials and biological mechanisms revisited. Eur J Oral Sci 2017; 125: 315–337.

Sonocoating with nHA and nZnO:Ag

The principle of sonocoating of objects with NPs in aqueous medium:

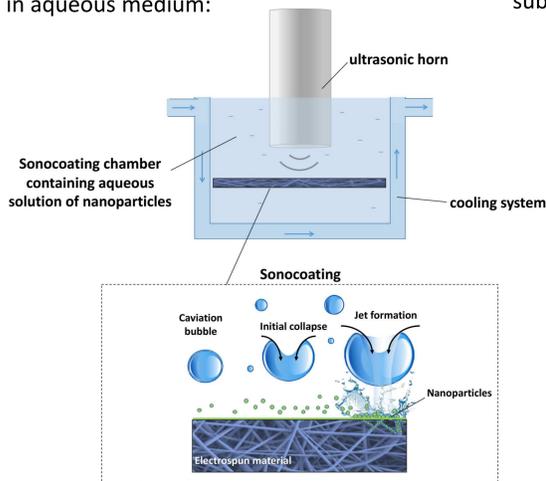


Fig.2 The scheme of sonocoating process. Source: Higuchi J, et al. Polymer Membranes Sonocoated and Electrospayed with Nano-Hydroxyapatite for Periodontal Tissues Regeneration, Nanomaterials 2019, 9, 1625; doi:10.3390/nano9111625.

Fiber morphology before and after subsequent coating with nHA and nZnO:Ag:

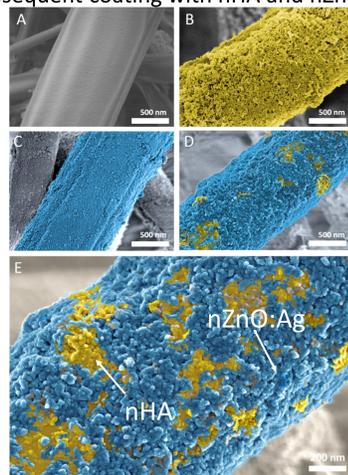


Fig.3 Colored SEM image of: A) non-coated PDLLA/PLGA fiber, B) PDLLA/PLGA/nHA, C) PDLLA/PLGA/nZnO:Ag, D) PDLLA/PLGA/nHA/nZnO:Ag after coating and E) PDLLA/PLGA/nHA/nZnO:Ag after 24h immersion in PBS medium at 37 °C.

Physicochemical properties

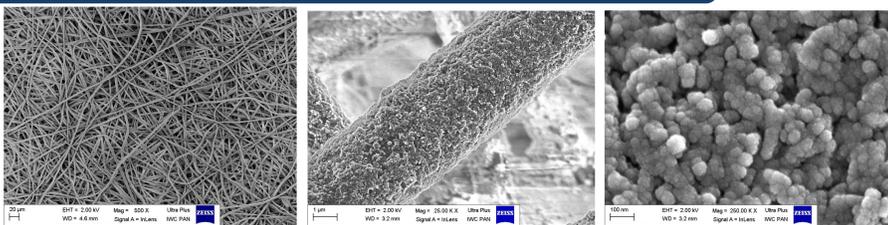


Fig.3 Colored SEM image of: A) non-coated PDLLA/PLGA fiber, B) PDLLA/PLGA/nHA, C) PDLLA/PLGA/nZnO:Ag, D) PDLLA/PLGA/nHA/nZnO:Ag after coating and E) PDLLA/PLGA/nHA/nZnO:Ag after 24h immersion in PBS medium at 37 °C.

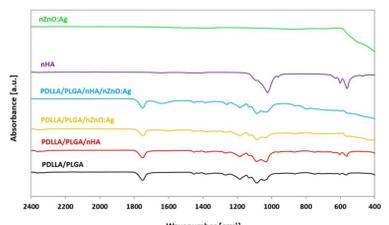


Fig.4 FTIR spectra of synthesized nanopowders (nHA, nZnO:Ag, electrospun polymer blend) and sonocoated samples.

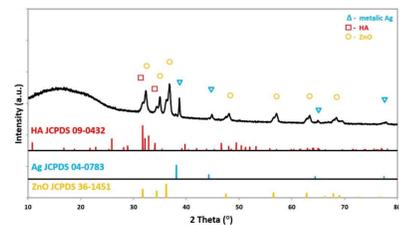


Fig.5 X-ray diffraction patterns of the PDLLA/PLGA/nHA/nZnO:Ag sample correlated with JCPDS data for HA, Ag, and ZnO.

References

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- Higuchi, J. et al. Electrospun Membrane Surface Modification by Sonocoating with HA and ZnO:Ag Nanoparticles—Characterization and Evaluation of Osteoblasts and Bacterial Cell Behavior In Vitro. *Cells* 2022, 11, 1582.
- Polish Patent PL240082B1 Rogowska-Tylman et al. Biological separative membrane, 14.02.2022.

Antibacterial activity

The inhibitory activity of membranes was assessed by measuring the inhibition zone diameter of the materials incubated with bacterial strains. The antibacterial activity of electrospun fiber mats was determined on *S. aureus* and *E. coli* strains. Sterile bacterial test strains were streaked on solidified agar plates to attain uniform growth. Test materials in the form of 6 mm-diameter discs were then placed on the plates and incubated for 24 h at 36 ± 2 °C. After incubation, microbial growth inhibition zones were determined (Fig.4). It was proved that materials containing nZnO:Ag layers show good antibacterial activity comparable to inhibition zones usually obtained for antibiotics. Moreover, the presence of nHA did not lower significantly the antibacterial activity of the material.

SAMPLE	BACTERIAL STRAIN	
	<i>E. coli</i>	<i>S. aureus</i>
PDLLA/PLGA	Inhibition zone = 0 mm	Inhibition zone = 0 mm
PDLLA/PLGA/nHA	Inhibition zone = 0.23 mm	Inhibition zone = 0.26 mm
PDLLA/PLGA/nZnO:Ag	Inhibition zone = 1.73 mm	Inhibition zone = 0.83 mm
PDLLA/PLGA/nHA/nZnO:Ag	Inhibition zone = 1.44 mm	Inhibition zone = 7.25 mm

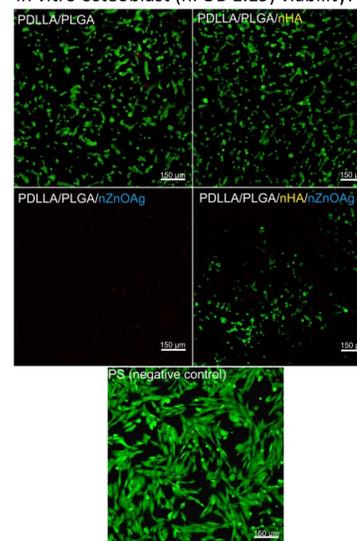
Fig.6 Representative SEM images and photographs of the bacterial inhibition zones formed by the different samples in contact with *Escherichia coli* and *Staphylococcus aureus* bacterial strains. The data are represented as mean \pm SD (n = 3).

In vitro bone cells response

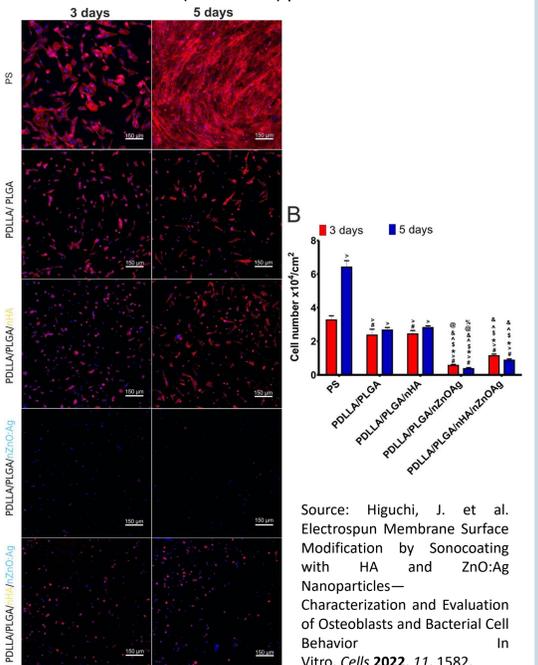
The biomaterial containing both nHA and biocidal nZnO:Ag provided more a favorable template for cell growth compared to the PDLLA/PLGA/nZnO:Ag sample, which indicates that the presence of an underlayer of nHA reduces the cytotoxic effect of nZnO:Ag.

The addition of the nHA underlayer to PDLLA/PLGA/nZnO:Ag made the surface of the biomaterial more suitable for osteoblast adhesion and growth after 3 and 5 days of incubation.

In vitro osteoblast (hFOB 1.19) viability:



In vitro osteoblast (hFOB 1.19) proliferation:

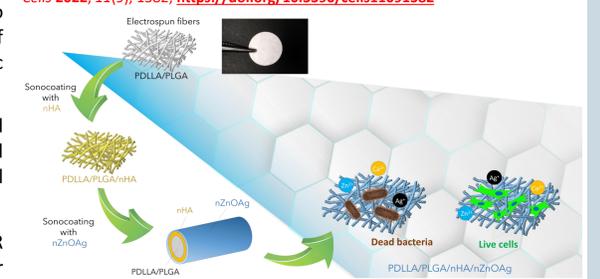


Source: Higuchi, J. et al. Electrospun Membrane Surface Modification by Sonocoating with HA and ZnO:Ag Nanoparticles—Characterization and Evaluation of Osteoblasts and Bacterial Cell Behavior In Vitro. *Cells* 2022, 11, 1582.

Summary

- It was proved that it is possible to precisely control sonocoating of nanoparticulate layers on thin polymeric microfibers.
- The sonocoated layer of nHA and nZnO:Ag on fibrous structure provided both bone cells growth and antibacterial activity against *S. aureus* and *E. coli*.
- It is a promising construct for GTR/GBR procedures but also all other applications of bone reconstruction.

Results were published recently in prestigious *Cells* journal (Impact Factor 7,7) *Cells* 2022, 11(9), 1582; <https://doi.org/10.3390/cells11091582>



Source: Higuchi, J. et al. Electrospun Membrane Surface Modification by Sonocoating with HA and ZnO:Ag Nanoparticles—Characterization and Evaluation of Osteoblasts and Bacterial Cell Behavior In Vitro. *Cells* 2022, 11, 1582.

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