Graphene-Loaded Bioactive Hydroxyapatite Coatings on Titanium Substrate – Fundamental *In Vitro* Investigations

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INTRODUCTION

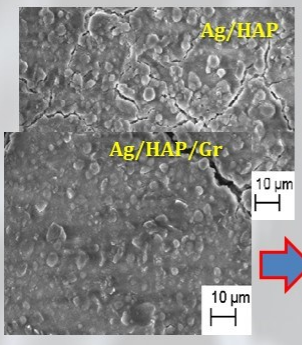
- One approach to enhance the bioactivity and improve biocompatibility of bone implant is by depositing bioactive thin films on Ti implant surface.
- Hydroxyapatite (HAP) is the major component of natural bone tissues known for its excellent biocompatibility and it is widely used in various forms and shapes in tissue engineering [1].
- Bacterial infection poses high risk factor for implant rejection. Therefore, inorganic HAP coating is doped with antibacterial agents, such as silver.
- Graphene (Gr) is well known for its excellent mechanical properties and very high specific surface area. Using Gr nanosheets as nanofillers could significantly improve the mechanical properties of bioactive coatings, but also retain HAP original biocompatibility [2].
- Electrodeposition (EPD) emerges as an attractive technique due to its simple set-up and formation of uniform coatings, even on substrates of complex shape [3].
- The aim was to explore potential of implementing Gr as HAP reinforcement, and depositing novel nanoscale composite silver/ hidroxyapatite/graphene (Ag/HAP/Gr) coatings on Ti substrate using EPD process.

EXPERIMENTAL

- Ethanol suspensions (pH = 2.00):
  c (Ag/HAP)= 1 wt. %, (obtained by modified chemical precipitation technique);
  c(Gr) = 0.01 wt. % (average thickness of the graphene nanoflakes - 12 nm, 30~50 layers of Gr monolayers);
  c (Ag) = 0.4 ± 0.1 wt. %.
- Working electrode: Ti plates (25 x 10 x 0.25 mm for surface analysis, 40 x 20 x 0.25 mm for impedance measurements, and 10 x 5 x 0.25 mm for cell based assays)
- Deposition conditions: U = 60 V for 2 min.
- Characterization techniques: XRD, FE-SEM, Raman spectroscopy, EIS, MTT test (cytotoxicity) and antibacterial activity kinetics in suspension.

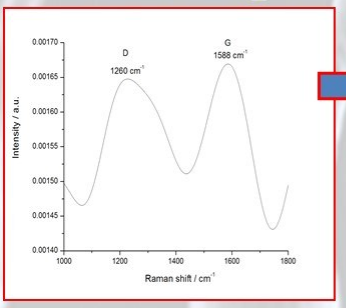
RESULTS AND DISCUSSION

FE-SEM



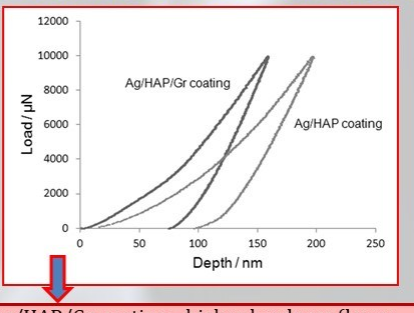
✓ Less cracked surface of Ag/HAP/Gr coating– Gr effectively acts as nano-reinforcement filler

Raman spectroscopy



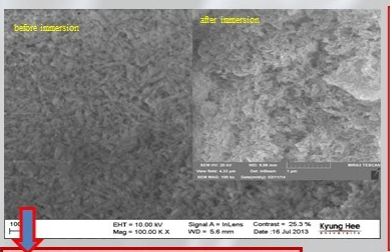
✓G-peak (~ 1588 cm^{-1}) ordered sp^2–bonded C atoms
✓D-peak (~ 1260 cm^{-1}) disordered aromatic structure or the Gr edges

Nanoindentation test

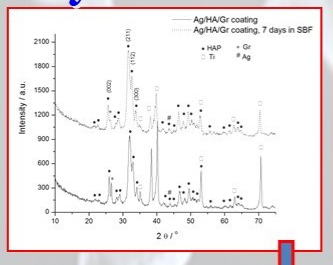


✓Ag/HAP/Gr coating - higher hardness (lower penetration depth) and higher reduced modulus (higher slope of the initial portion of the unloading curve)

Bioactivity

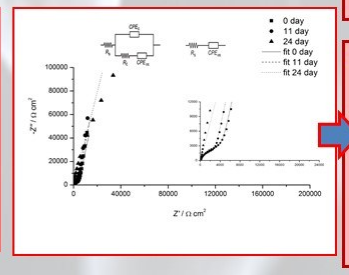


✓Rod-like individual grains of Ag/HAP/Gr coating (<50 nm).
✓Newly formed plate-shaped apatite layer after SBF
✓Highly porous surface - better cell adhesion



✓ Incorporation of Gr – $2\theta = 26.6^{\circ}$
✓ Increase in crystallite size after SBF (17.6 to 22.3 nm) - incorporation of CO_3^{2-} ions (bone-like HAP layer)

EIS



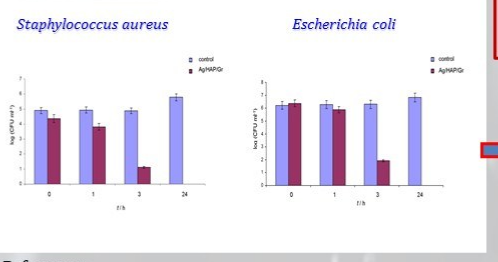
✓R_c values started to increase after 7 days - the beginning of new apatite layer
✓After 21 days (R_c = 73.9 kΩ cm^2) newly formed carbonated HAP

Cytotoxicity

Cell type	Peripheral blood mononuclear cells (PBMC)
Material	Ag/HAP/Gr coating
Cell viability (*S*), %	79.6 ± 11.2
Classification	Non-cytotoxic*

✓Mild decrease in survival of PBMC compared to the control cell sample (S = 100 %)

Antibacterial effect



✓ NO BACTERIAL GROWTH - reduction of *S. aureus TL* and *E. coli*
✓ After 24 h both – no viable cells and visible colonies

CONCLUSION

- ✓Using EPD the biocomposite Ag/HAP/Gr coatings were successfully deposited on Ti substrate.
- ✓ An addition of Gr into Ag/HAP coating significantly improved its morphology, composition, mechanical properties, and bioactivity compared to graphene-free Ag/HAP coating.
- ✓ Ag/HAP/Gr coatings exhibited strong antibacterial activity against *S. aureus* TL and *E. coli*, therefore suppressing biofilm formation.
- ✓ Cytotoxicity - Ag/HAP/Gr coatings were classified as non-cytotoxic within the margin of error, against target PBMCs.

References:
[1] M. Stevanović, M. Đošić, A. Janković, V. Kojić, M. Vukašinović-Sekulić, J. Stojanović, J. Odović, M. Crevar Sakač, K. Y. Rhee, V. Mišković-Stanković, *ACS Biomater. Sci. Eng.* 2018 **4** (12),3994-4007..
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[3] M. Stevanović, M. Djošić, A. Janković, V. Kojić, M. Vukašinović-Sekulić, J. Stojanović, Jadranka Odović, Milkica Crevar Sakač, Rhee Kyong Yop, Vesna Mišković-Stanković, *J. Biomed. Mater. Res. - Part A.* **108**(11) (2020) 2175-2189.

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