Bioactive peptide loading nanoparticles for the inhibition of the local inflammatory microenvironment

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Abstract

Modifications to titanium dental implants have surged to improve biomimetic conditions and encourage bone growth. However, activated pro-inflammatory macrophages in the local inflammatory microenvironment have inhibitory effects on bone regeneration. This study explored whether coating RGD and TCP-25 with nanoparticles could influence the regulation of the inflammatory response governed by macrophages. Herein, mice macrophages were polarized into their pro-inflammatory or pro-regeneration phenotype and cultured in titanium substrates with either ZIF-8 nanoparticlesor ZIF-8 nanoparticles encapsulated in TCP-25 and RGDpeptides. Then, the gene expression levels of GAPDH, MAPK, Taz, TGFβ, and Smad3 were studied. We illustrated the signaling pathways associated to these proteins in macrophages. **Our findings demonstrated that the TCP-25/RGD@ZIF-8 nanoparticles can significantly suppress the pro-inflammatory polarization and promote the pro-regeneration polarization of the seeded macrophages via the MAPK and TGFβ/Smad3 signaling pathway.** This will give insight for future studies on how to direct M1 to M2 polarization on titanium substrates.

Background

- Titanium dental implants induce inflammatory reactions, which polarize macrophages to their M1 state.
- Nanoparticles pose a slow-releasing delivery system for encapsulated peptides.
- The TCP-25 peptide inhibits Toll-like receptor 4 (TLR-4), which decreases inflammatory response.
- RGD helps with cell adhesion, spreading, and pro-regenerative M2-type polarization.

Methods

ZIF-8 nanoparticles were synthesized through a reaction between 2-methylimidazole and zinc nitrate. ZIF-8 nanoparticles were encapsulated with arginylglycylaspartic acid (RGD) and thrombin-derived C-terminal peptide (TCP-25). H-NMR was performed on the nanoparticles to confirm successful encapsulation. Naive macrophages were cultured in minimum essential media (MEM) and bovine serum albumin. These were divided into M1 and M2 groups by transferring them to M1 induction medium with LPS and IFN-γ and M2 induction medium with IL-13 and IL-14. RT-qPCR was performed to detect the gene expression of GAPDH, MAPK, Taz, TGFβ, and Smad3. Immunoflourescence was performed to detect the expression of CD-90 and Integrin-β1.

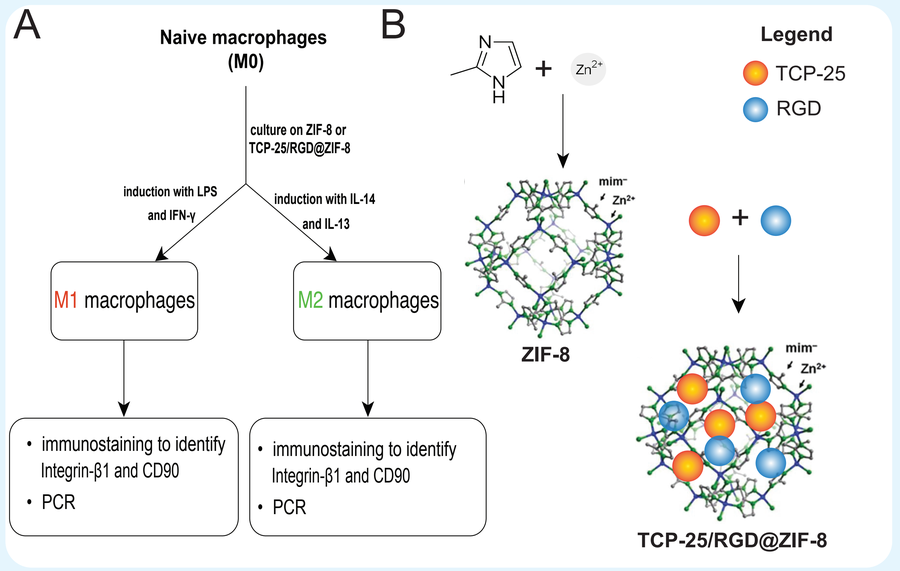

Figure 1. A) Workflow. B) Scheme of the encapsulation of the ZIF-8 nanoparticles.

Results

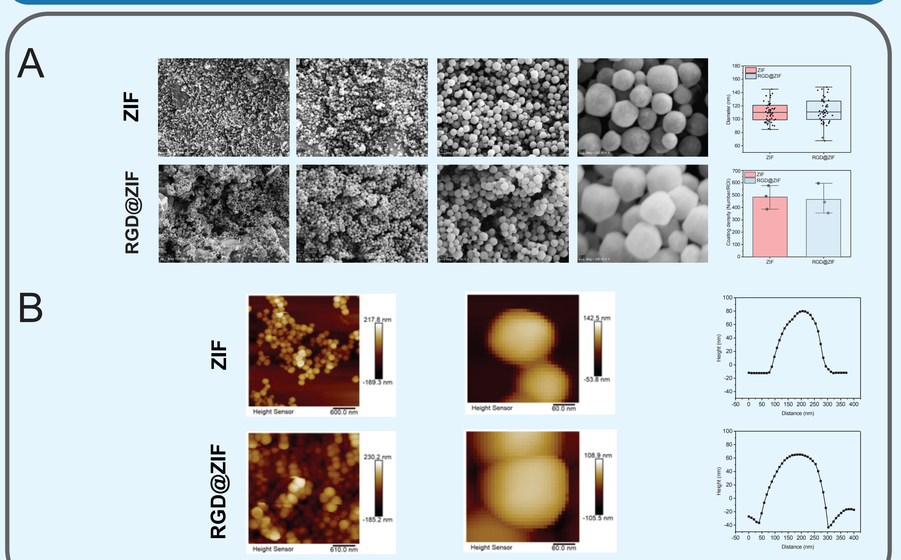

Figure 2. SEM and AFM characterization of NPs

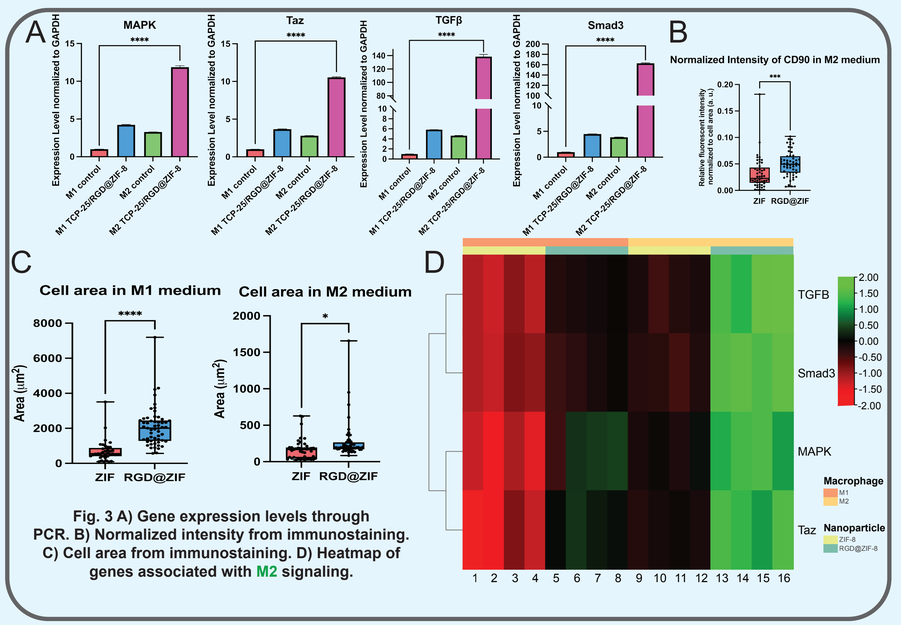

Fig. 3 A) Gene expression levels through PCR. B) Normalized intensity from immunostaining. C) Cell area from immunostaining. D) Heatmap of genes associated with M2 signaling.

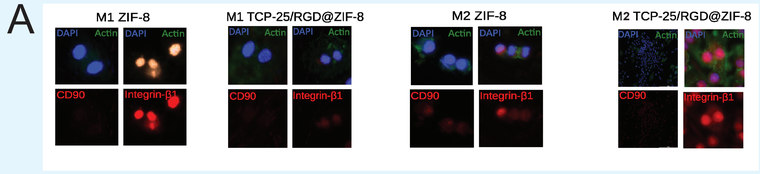

Figure 5. Immunofloruescence staining

Students t-test revealed higher levels of CD90 in the macrophages cultured in the M2 medium with TCP-25/RGD@ZIF nanoparticles (Fig. 3). One-way ANOVA ($p<0.05$) was used to identify differences in average gene expression obtained from PCR results (Fig. 3). In M1 and M2 macrophages, cell area was higher in the groups with TCP-25/RGD@ZIF-8 (Fig. 3). TGF-β may have stimulated cell spreading and adhesion, showing higher cell area in the macrophages treated with RGD and TCP-25 (Fig. 3). TGF-β, Taz, MAPK, and Smad3 were upregulated the most in the samples cultured in M2 induction media that had interacted with TCP-25/RGD@ZIF-8 (Fig. 3).

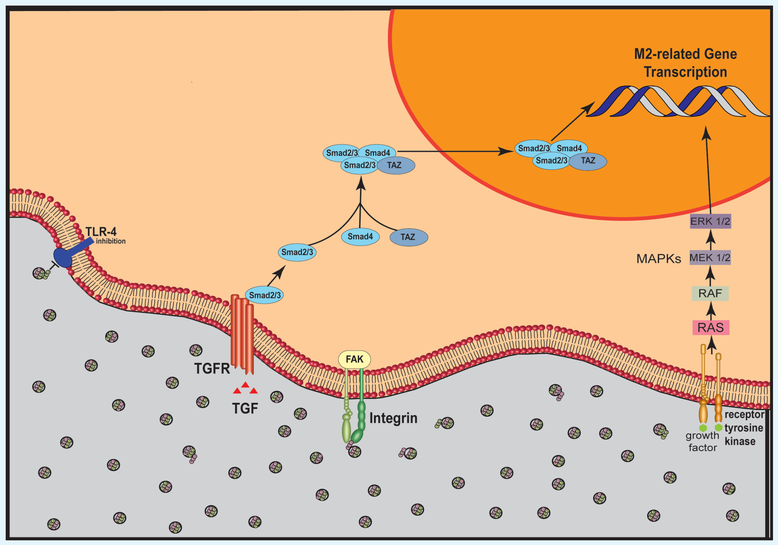

Figure 6. M2-related gene transcription is pro-regeneration and adhesion.

Conclusions

The macrophages that interacted with TCP-25/RGD@ZIF-8 in titanium substrate had a higher expression of TGFβ, MAPK, Taz, and Smad3 in comparison with those that interacted with ZIF-8 only. Since these genes are related to the M2 anti-inflammatory and pro-regeneration phenotype, the encapsulation of the ZIF-8 with TCP-25/RGD@ZIF-8 increased polarization to the M2 phenotype.

References

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