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Predictive and experimental study of intelligent nanoparticles for controlling cardiac ECM after myocardial infarction

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Introduction

Recent advancements in nanotechnology have the potential to revolutionize both preventive and therapeutic approaches for treating cardiovascular disease [1]. Nanoengineering approaches could be exploited to develop scaffolds recapitulating the complex signals necessary for cardiac regeneration. Recently, cardiac scaffolds were modified with nanoparticles (NPs) able to recognize a specific ECM metalloproteinase (MMP) with the aim to prevent left ventricular (LV) dysfunction after myocardial infarction (MI) [2]. It is known that an increased LV myocardial MMP activity and selective upregulation of MMPs in heart failure occur. The control of MMP activation and expression in the failing human LV myocardium represents a significant therapeutic target for heart disease. Our idea was to exploit the molecular imprinting polymers (MIP) in order to restore the correct MMPs/TIMPs balance. In this work, we have explored more in depth the potential of this technique through a wide scenario of evaluations from the computational study to the synthesis and physico-chemical, dimensional and biological characterization.

Experimental Methods

Methacrylic acid (MAA), poly(ethylene glycol) ethyl ether methacrylate ((PEG)EEMA) as monomers and trimethylolpropane methacrylate (TRIM) as cross-linker were used. Human MMP-9 was used as template. MD simulations were performed by using the AMBER16 software employing the General Amber Force Field (GAFF) for MAA and (PEG) EEMA species and the ff14SB Force Field for the MMP-9 protein. The structure of isolated MMP-9 protein was extracted from PDB database. MIP NPs were synthesized using precipitation radical polymerisation of MAA and (PEG)EEMA with monomer ratio 75/25 (selected from the computational analysis) with a low coverage of the protein. FT-IR Chemical Imaging was used to evaluate the chemical map of pure MMP-9 and NP aggregates before and after template extraction. DLS analysis was carried out on different NP aqueous dispersions. SEM analysis was performed on both MIP NPs and control NPs. HPLC analysis was performed to evaluate rebinding capacity and selectivity of MIP NPs towards MMP-9. Viability assay was carried out on H9c2 cardiomyoblasts incubated with increasing MIP NPs concentrations at 24, 48 and 72 hrs through Propidium Iodide Flow Cytometry analysis.

Results and Discussion

The evolution of the interaction between MMP-9 and MIP NPs as a function of monomer concentration was evaluated by a new and original computational protocol. A stronger water/protein interaction than the monomers/protein one was observed, although, in the case of low coverage, this effect was reduced especially for (PEG)EEMA monomers that resulted more strongly anchored to the protein surface. FT-IR

Chemical Imaging allowed to identify a diagnostic band for MMP-9 and detect this band both before and after template extraction from MIP NPs. An interesting similarity between a partial coverage where the enzyme structure is exposed respect to monomer shell and chemical map showing a surface presence of enzyme on NPs was observed. An high percentage of MMP-9 entrapped into NPs was evaluated by HPLC confirming the strong interaction protein/NPs and an elevated enzyme extraction. SEM analysis showed an average value of sphere dimension only slightly higher for MIP NPs than control NPs. This result was confirmed by DLS analysis indicating that surface sites can take place allowing the removal of MMP-9 after imprinting. HPLC analysis showed a rebinding capacity of MIP NPs towards the enzyme confirming the presence of highly-specific recognition zones in agreement with the computational study. MIP NPs exhibited also selectivity towards MMP-9 respect to an analogue enzyme (MMP-2). Finally, no cytotoxic activity of MIP NPs was observed on H9c2 cells: the viability values were close to 100% independently of dose and incubation time.

Conclusion

Molecular imprinting was proposed for the production of nanoparticles capable of modulating enzymes of the MMP family for restoring the correct MMPs regulation in the cardiac microenvironment. This study aims to underline the importance of a comprehensive study, using a large and synergic set of analyses and methodologies, to better explore and imitate the complex interactions between engineered nanosystems and native tissue for progress in myocardial regeneration.

Acknowledgement

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References

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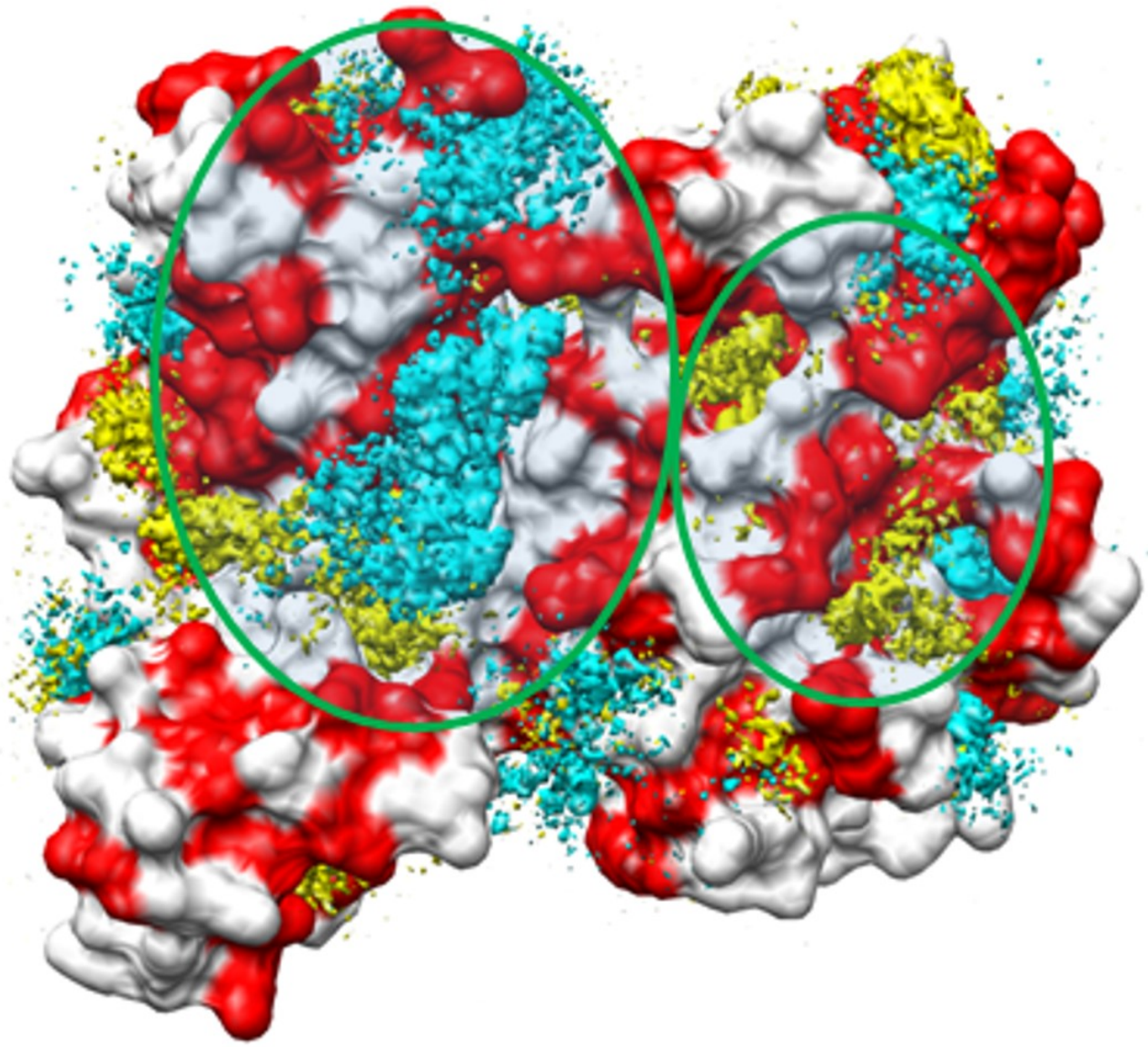
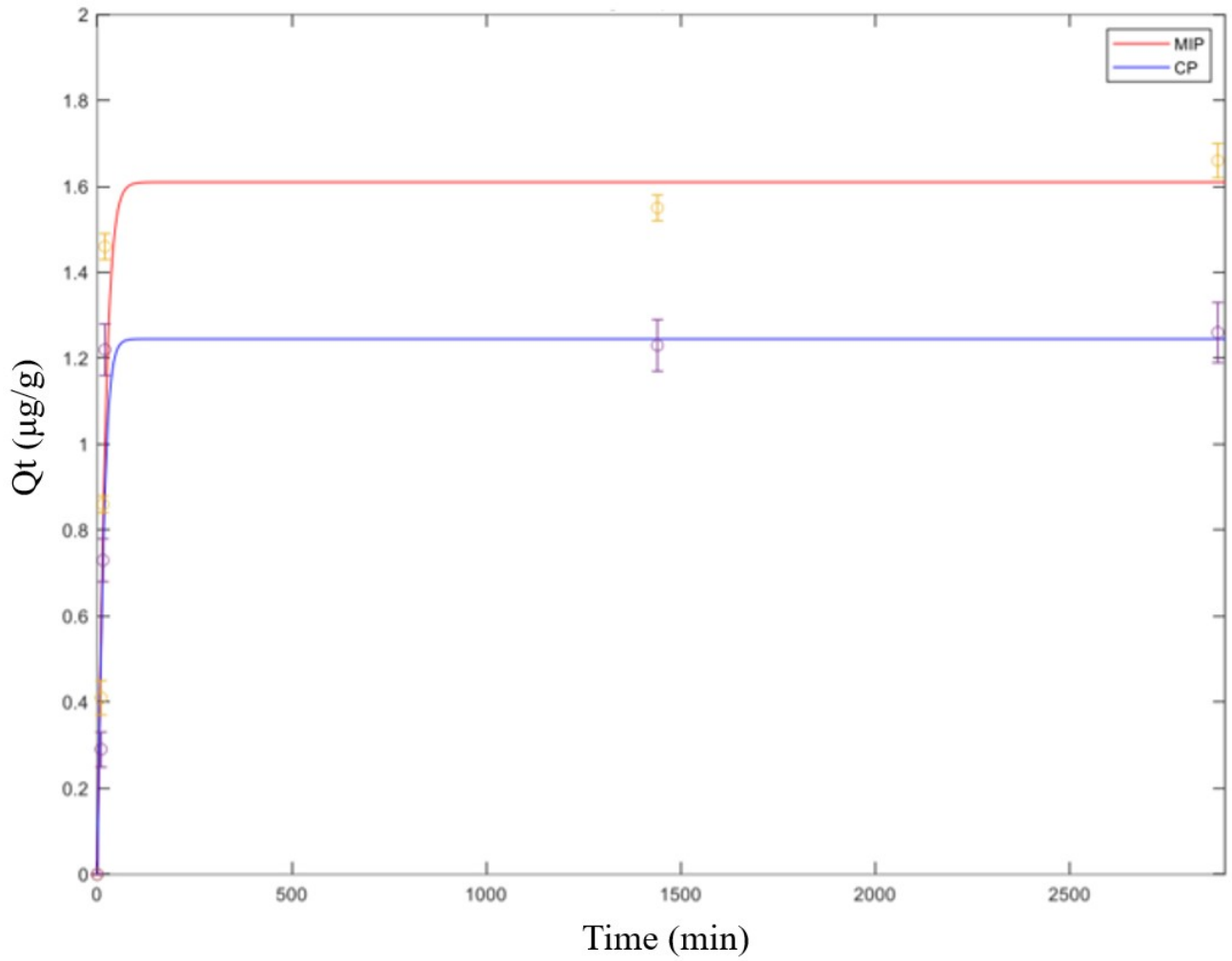


SDF of monomers around the MMP-9
Special distribution functions (SDF) of the MAA (yellow) and (PEG)EEMA (cyan) monomers around the MMP-9 protein in the presence of water.



Rebinding MMP-9: MIP vs CP

MMP-9 rebinding assay of MIP and control NPs (CP).