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SUPPORTING INFORMATION

Microbial Transglutaminase nanoflowers as an alternative nanomedicine for breast cancer theranostics

Syeda Warisul Fatima[1], Khalid Imtiyaz[2], Mohammad M. Alam Rizvi[2], Sunil K. Khare[1,]*

[1] Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, New Delhi-110016, India

[2] Genome Biology Laboratory Department of Biosciences, Jamia Millia Islamia, New Delhi-110025, India

*Correspondence: skkhare@chemistry.iitd.ac.in, Tel. +91-112659 6533, +91-112659 6568

https://orcid.org/0000-0002-7339-3058;

Transglutaminase Assay

The MTGase was assayed by using Z-Gln-Gly as substrate following the method of Folk and Cole (1965). Briefly, to the substrate mixture (1.5 ml) consisting of Z-Gln-Gly (37.5 mM), hydroxylamine (125 mM) in glutathione reduced form (12.5 mM) and $CaCl_2$ (1000 mM), 0.5 ml MTGase enzyme was added and incubated at 37 °C for 10 min. The reaction was terminated by adding 1.5 ml stopping reagents (5% $FeCl_3$ (w/v), 12% (v/v) in Trichloroacetate, 0.8 M HCl) followed by recording absorbance at 525 nm. One unit of MTGase was defined as the amount of enzyme which causes the formation of 1.0 µmol L-Glutamic acid γ- monohydroxamate per minute at 37 °C. The colorimetric enzyme assays were performed by using UV 1601 spectrophotometer (Shimadzu, Kyoto, Japan).

Moreover, Transglutaminase when used in nanoflower form, exhibited an increment in its activity, 156.74 % fold of that of free enzyme (3.14 IU/ml) along with displaying effective durability and reusability, highlighting its potential for repeated uses. It was found that their activity was retained even after tenth cycle of reuse. Significant improvement in kinetic parameters (catalytic efficiency was found to be 6.92 fold higher than free MTGase).

Table S1. Kinetic Parameters of Free Transglutaminase enzyme and nanoflowers form

Kinetic Parameter of Transglutaminase enzyme	Free form (Control)	Nanoflowers form	Fold Change
Vmax (mM min^{-1})	10.69	19.03	1.78 ↑
Km (mM)	14.78	3.80	0.26 ↓
Catalytic Efficiency	0.72	5.01	6.92 ↑

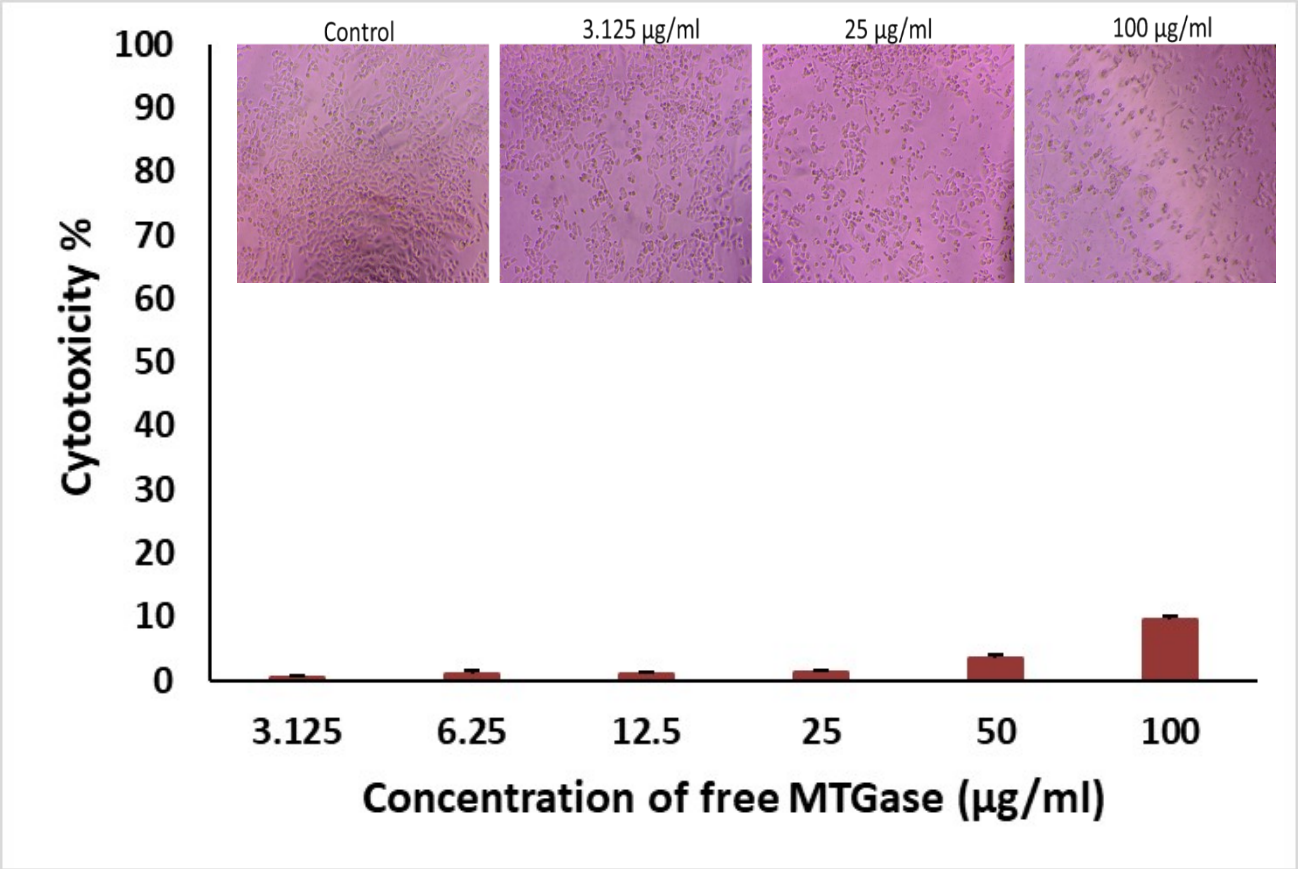

Fig. S1 Cytotoxicity assessement induced by microbial Transglutaminase free enzyme on breast cancer (MCF-7) cell line at 24 h post-treatment; (inset-microscopic images after treatment for MTT free form of enzyme MTGase did not show a significant effect as compared to the nanoflowers)

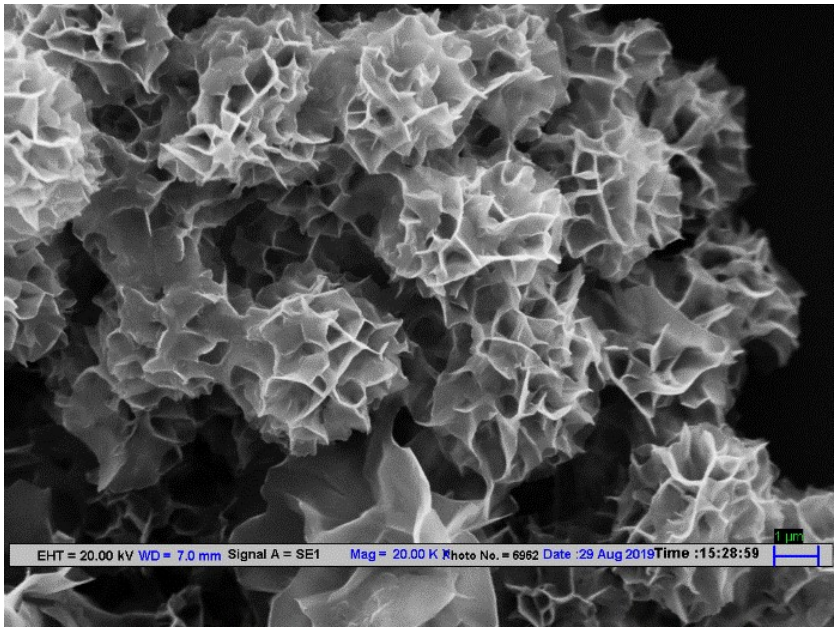

Fig. S2 shows the intact MTGase nanoflowers by SEM after withstanding harsh environmental condition (solvent). The robustness of these enzymatic nanoflowers denotes the resistance capacity owing to its inherent nature of providing protecting due to iso-peptide cross-linking.

References

1 J.E. Folk and P.W. Cole, Structural requirements of specific substrates for guinea pig liver transglutaminase. J. Biol. Chem., 1965, 240, 2951-2960.