Polyphenol-Enabled 2D Nanopatch for Enhanced Nasal Mucoadhesion and Immune Activation

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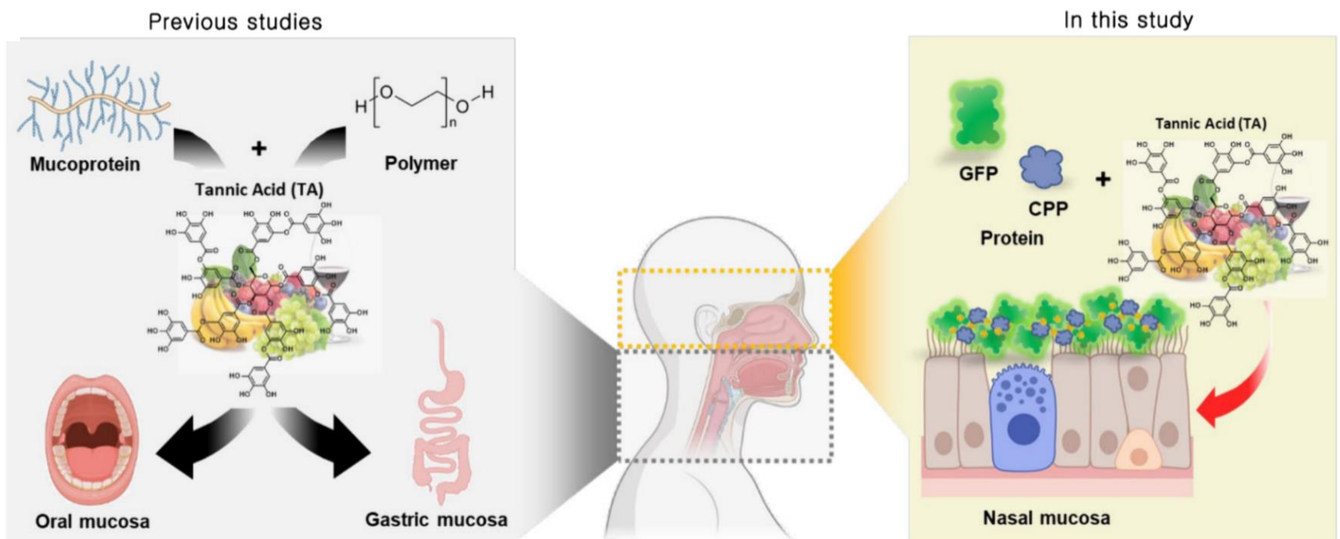
Abstract



The advancement of effective nasal mucoadhesive delivery faces challenges due to rapid mucociliary clearance (MCC). Conventional studies have employed mucoadhesive materials, mainly forming spherical nanoparticles, but these offer limited adhesion to the nasal mucosa. This study hypothesizes that a 2D nanoscale structure utilizing adhesive polyphenols can provide a superior strategy for countering MCC, aligning with the planar mucosal layers. We explore the use of tannic acid (TA), a polyphenolic molecule known for its adhesive properties and ability to form complexes with biomolecules. Our study introduces an unprecedented 2D nanopatch, assembled through the interaction of TA with green fluorescent protein (GFP), and cell-penetrating peptide (CPP). This 2D nanopatch demonstrates robust adhesion to nasal mucosa and significantly enhances immunoglobulin A secretions, suggesting its potential for enhancing nasal vaccine delivery. The promise of a polyphenol-enabled adhesive 2D nanopatch signifies a pivotal shift from conventional spherical nanoparticles, opening new pathways for delivery strategies through respiratory mucoadhesion.

Results

Complex of GFP/CPP/TA showed 2D flat nanopatch structure

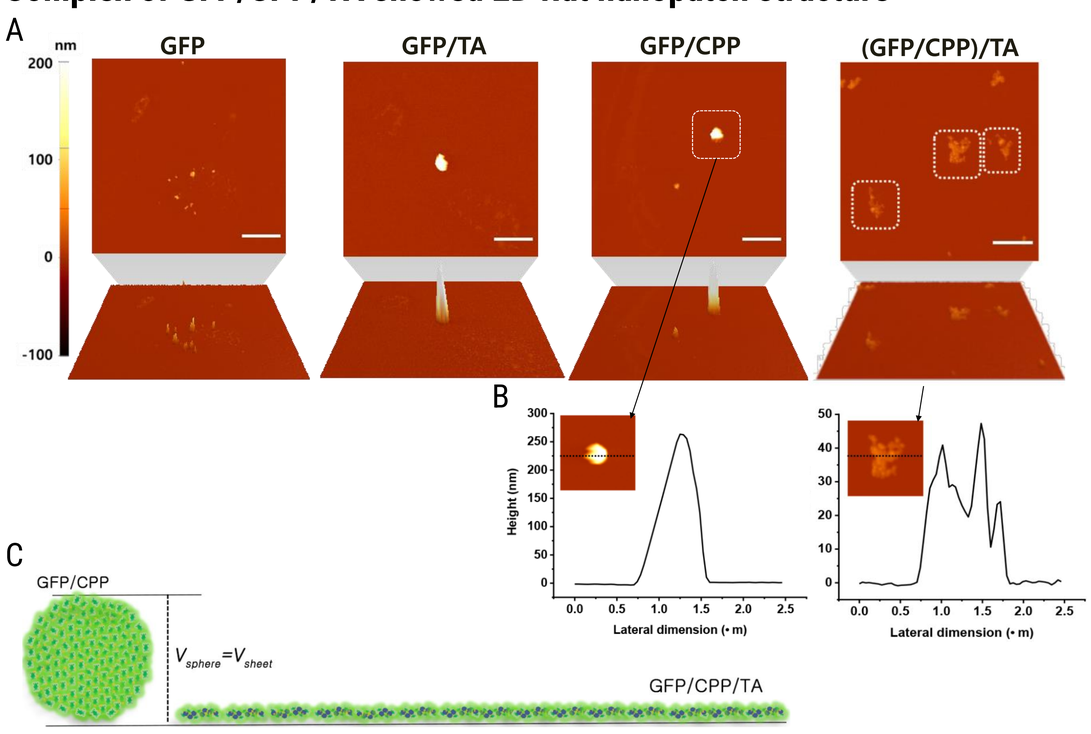


Figure 1. (A) AFM imaging of GFP only, GFP/CPP mixture, GFP/TA mixture, and GFP/CPP/TA complex. (B) Representative line profile of GFP/CPP mixture particle. GFP/CPP/TA complex showing a 2D flat structure and its line profile. (C) A schematic description showing the structural shift from spherical to 2D nanopatch, maintaining similar volume (V_{sphere} = V_{patch}).

2D nanopatch showed mucoadhesive and cell adhesive character

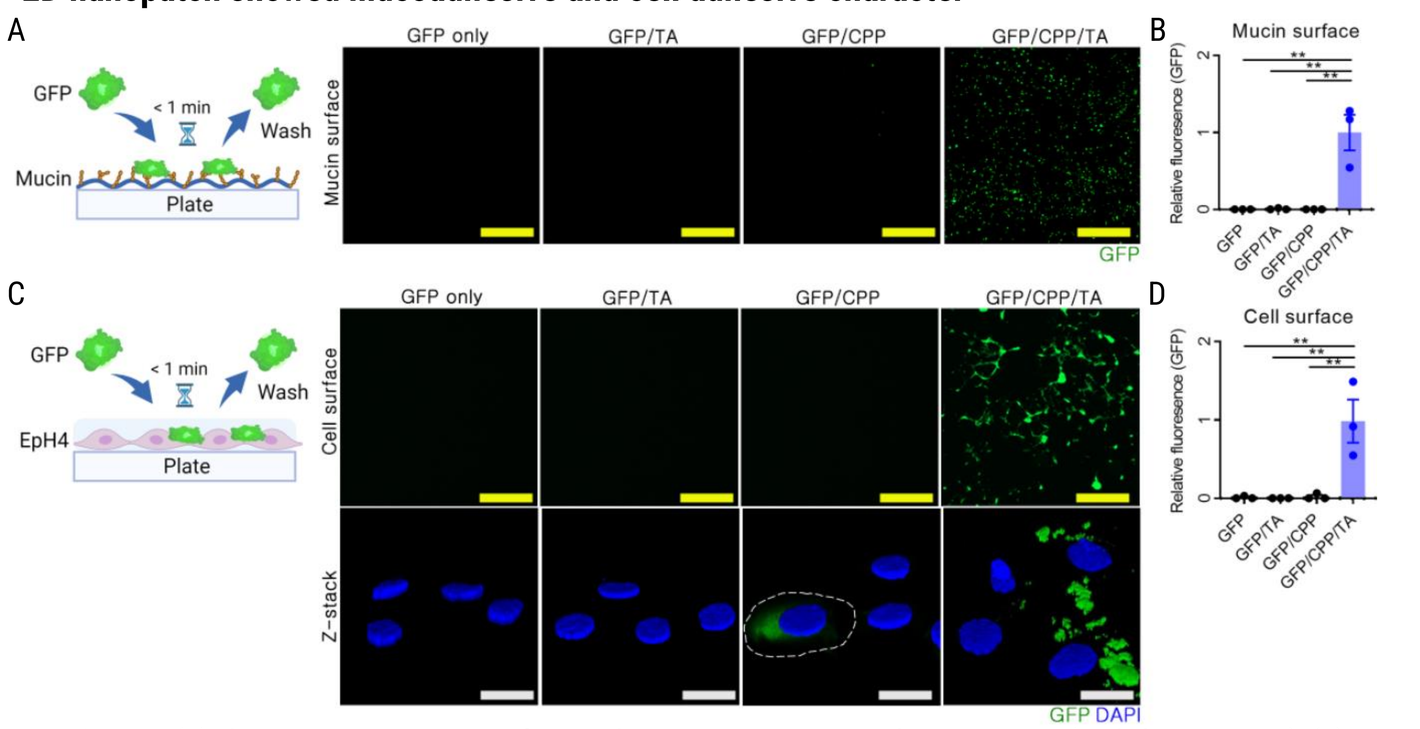
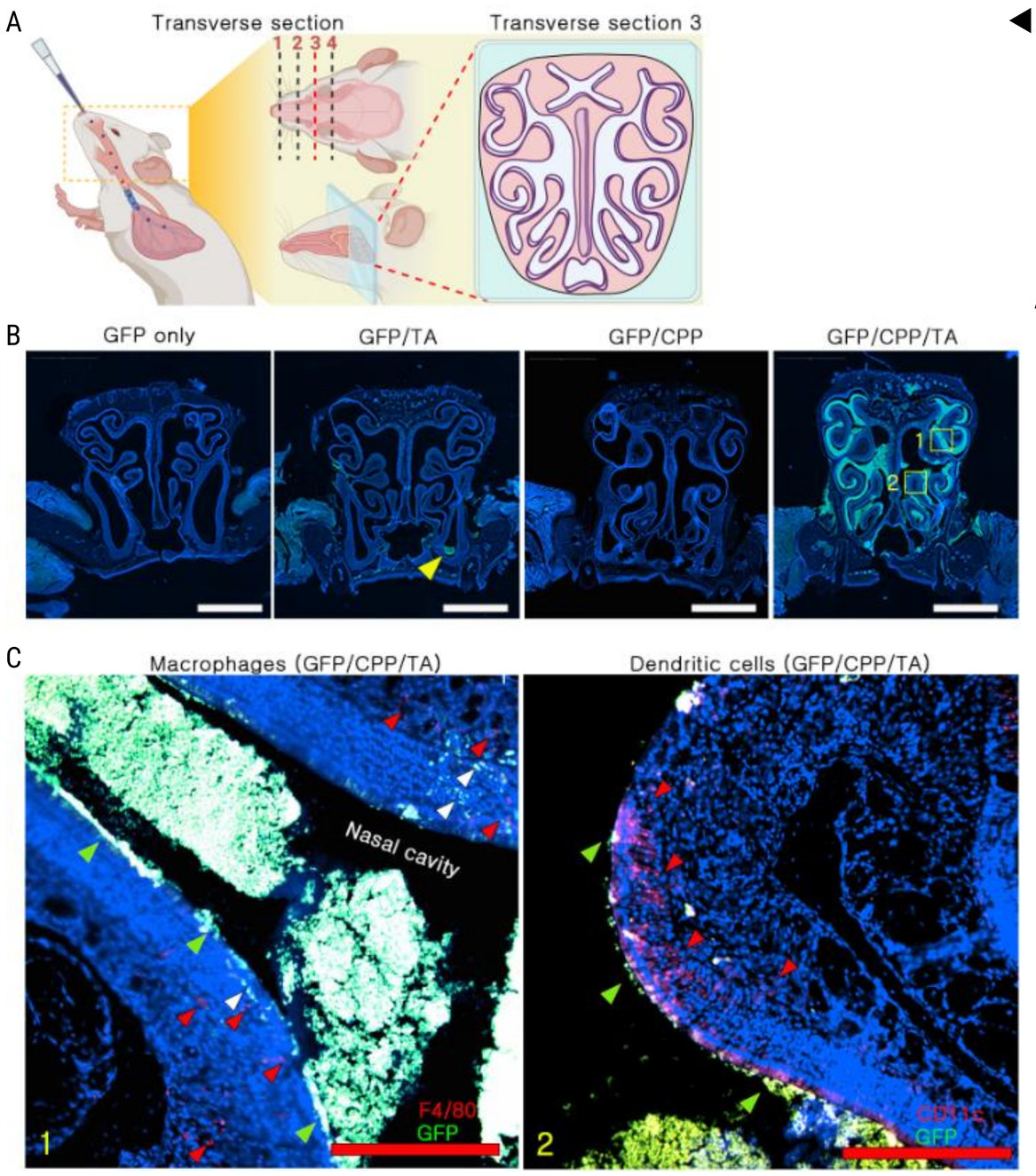


Figure 2. (A) Fluorescence microscopy of GFP only, GFP/TA, GFP/CPP, and GFP/CPP/TA. (scale bar = 300 μm). (B) Quantitative analysis of GFP signal intensity using ImageJ software. The measured GFP intensities from each group were normalized to the average GFP/CPP/TA values. (C) Fluorescence microscopy of analyzing adhesion to cell surfaces (scale bar = 400 μm) and Z-stack confocal microscopy GFP (green) and DAPI (blue) visualize proteins and nuclei, respectively, with cell membranes marked by dashed white lines (scale bar = 20 μm). (D) Quantification of GFP signal was analyzed using the same method as (B).

2D nanopatch showed nasal mucoadhseion and immune activation



◀ **Figure 3**. (A) Brief scheme for intranasal injection process and preparation of nasal cavity section. The nasal cavity section was conducted with a transverse section at the second palatal ridge (transverse section 3). (B) Fluorescence microscopy providing mucoadhesive GFP from nasal cavity sections 2 h after intranasal injection (scale bar = 2000 μm). Yellow triangle indicated remained GFP. (C) Immunofluorescence staining for murine macrophages [1] and murine dendritic cells [2]. Red triangles indicated F4/80 positive macrophages or CD11c positive dendritic cells. Green triangles indicated mucous layer adhered GFP. White triangles indicated cell penetrated to submucosal layer. Scale bar = 200 μm.

2D nanopatch enhanced igA production without adjuvant

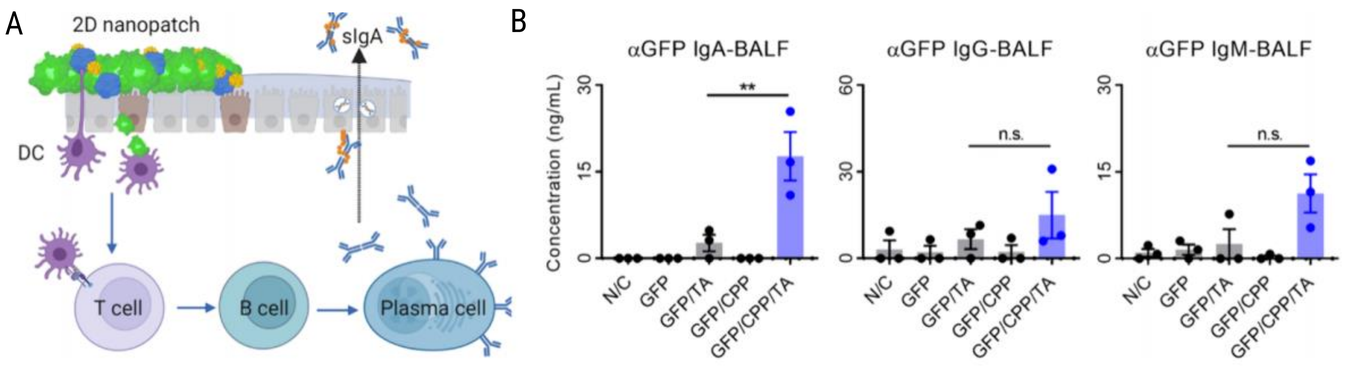


Figure 4. (A) A schematic image for immunoglobulin A (IgA) production by 2D nanopatch (B) Quantification of anti-GFP IgA, IgG, and IgM levels in bronchoalveolar lavage fluid (BALF), measured by ELISA

Conclusion

- This study developed a novel polyphenol-enabled 2D nanopatch composed of GFP, CPP, and TA.
- The nanopatch adheres robustly to nasal mucosal surfaces and significantly enhances mucosal IgA secretion.
- The biodegradable nanopatch effectively promotes nasal mucosal immunity and overcomes challenges posed by mucociliary clearance.
- It addresses the limitations of traditional spherical nanoparticles, paving the way for new strategies in nasal mucosal delivery and improved respiratory disease prevention.

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