Layer-by-Layer Nanofabrication to Orchestrate Interplay between Macrophage and Stem/Progenitor Cells

COLUMBIA | COLLEGE OF DENTAL MEDICINE

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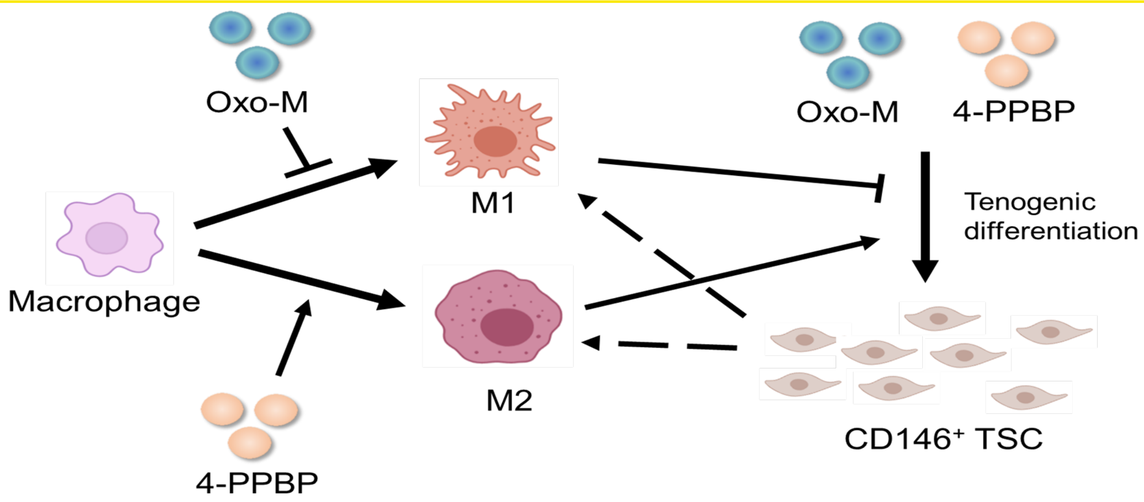
BACKGROUND

Interplay between stem/progenitor cells and macrophages is involved in the tissue healing and regeneration processes. For a timely orchestration of macrophage polarization and stem/progenitor cell bioactivities, we applied layer-by-layer (LbL) nanofabrication to provide precise control of time, duration, and sequential release of two small molecules (SM), Oxo-M and 4-PPBP, given their distinct functions in differentiating stem/progenitor cells and macrophage polarization.

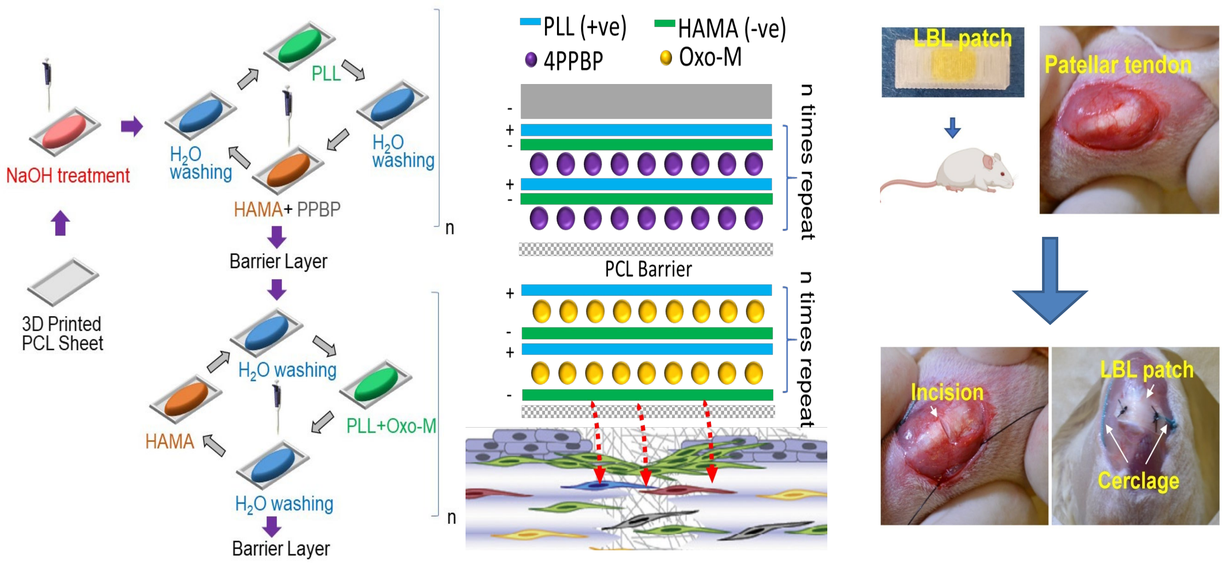


OBJECTIVES

1. Optimize nano-coating thickness of PAH/MA-HA by regulating polymer concentration and coating time
2. Determine the loading capacity and release profile of SM from PCL patch with LbL nano-coating
3. Investigate bioactivities of released SM from the PCL patch with LbL nano-coating.

METHODS & MATERIALS

The LbL nano-coatings were made on a 3D-printed polycaprolactone (PCL) patch, using a repeat of sequential deposition of cationic polymer poly-l-lysine (PLL) and anionic polymer methacrylated hyaluronic acid (HAMA), where Oxo-M or 4-PPBP was loaded between the layers. Confocal microscopy was used to examine the thickness and distribution of the nanolayers, in vitro release was measured, and bioactivities of released SM were tested by culturing tendon stem/progenitor cells (TSC). The SM-loaded LbL PCL patch was then applied to our well-established patellar tendon repair model.



RESULTS

Each polymer layer has 75 nm of thickness. Two distinct stacks of positive and negative polymers showed the coating uniformity, and the (PLL/HAMA)40 layered PCL patch with sequential loading of 4-PPBP and Oxo-M showed a fast release of Oxo-M by 7 - 10 days, followed by slow release of 4-PPBP up to 42 days. Scleraxis (SCX) and Mohawk (Mkx) expressions were significantly higher in Oxo-M/4-PPBP loaded LbL patches than in the single SM-loaded samples or no SM controls. In vivo, SM-releasing LbL PCL patches greatly enhanced tendon healing.

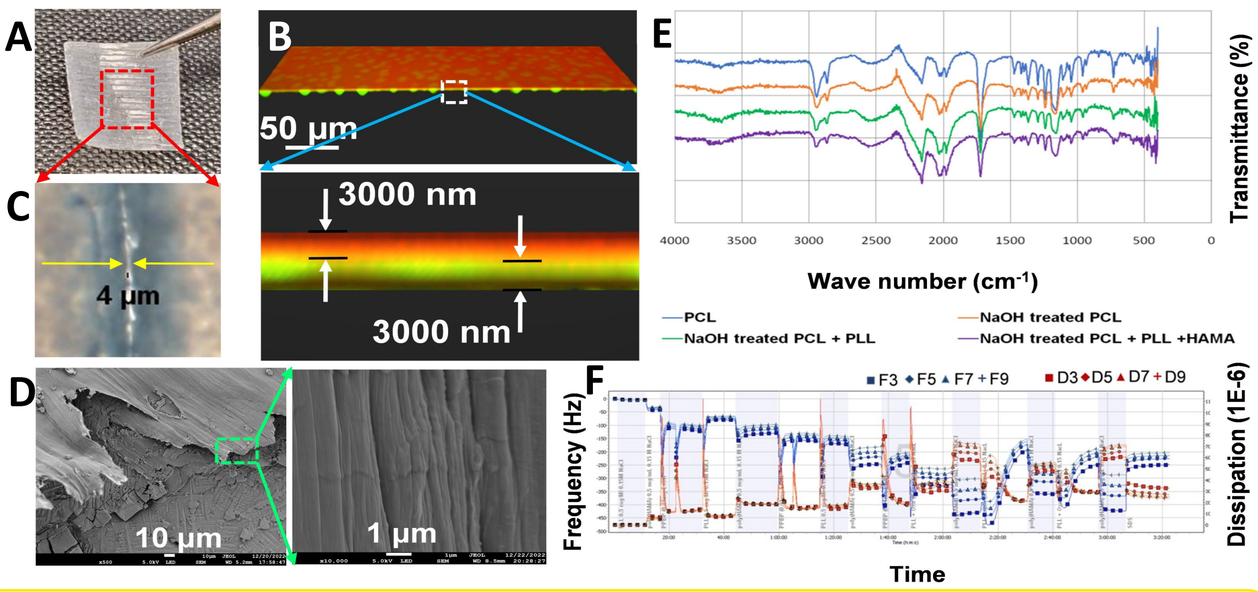


Fig. 1. Characteristics of nano-LbL patch (**A**) showing 3 μm-thick coating of 24 nano-layers (**B**) with 4 μm porous barrier (**C**). SEM images show multiple nano-layers (**D**) and FTIR (**E**) and QCM (**F**) confirm the multiple nano-layers.

RESULTS

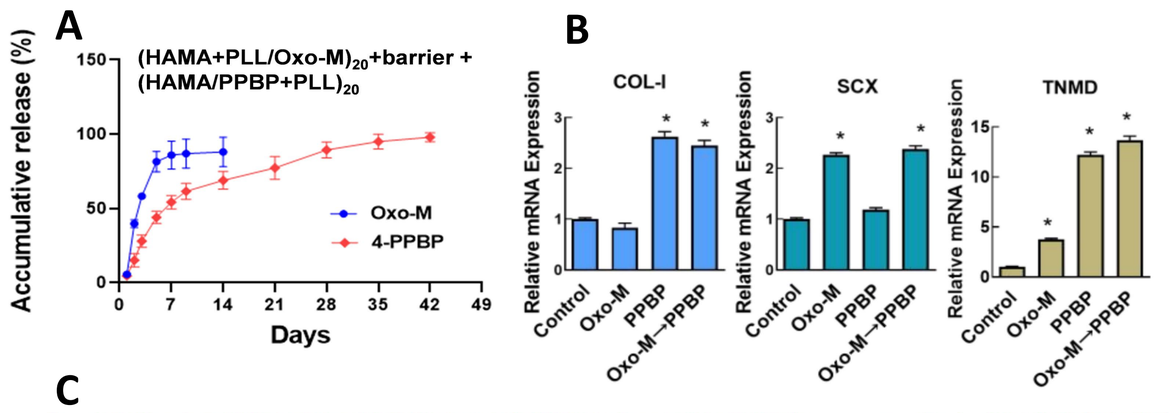


Fig. 2. Release kinetics of Oxo-M and 4-PPBP from LbL patch (**A**). Bioactivities of released OP from LbL patch tested with TSCs (**B**) (*p<0.05 compared to control; n = 5 per group). Live/dead assays with different doses of Oxo-M and 4-PPBP (**C**).

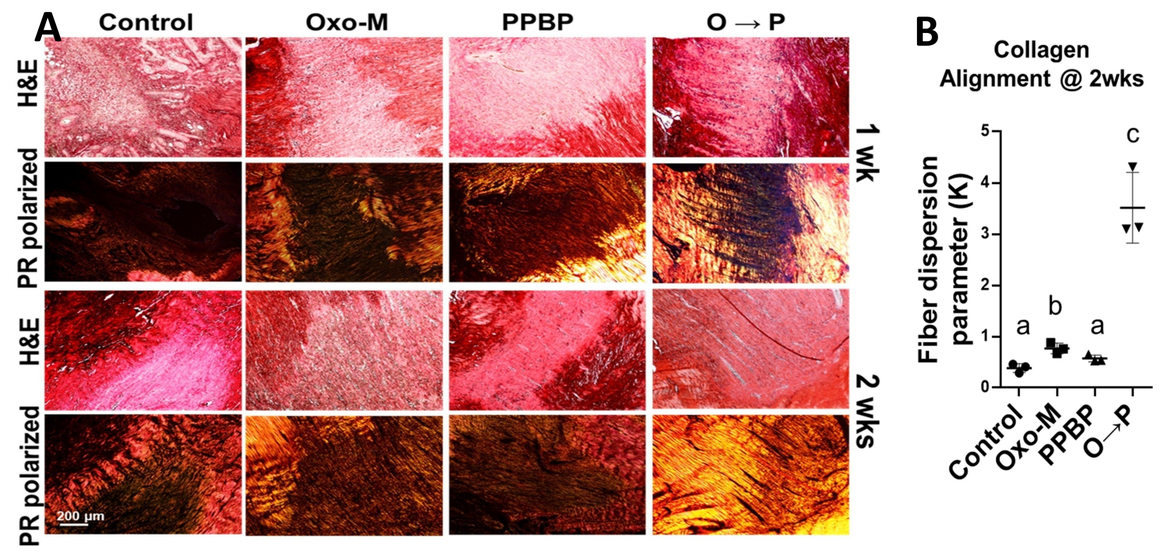


Fig. 3. Histological analysis show significant improvement in tendon healing (A), with significantly improved fiber orientation (B) (p<0.001; n = 15 per group; different letters indicate significant difference).

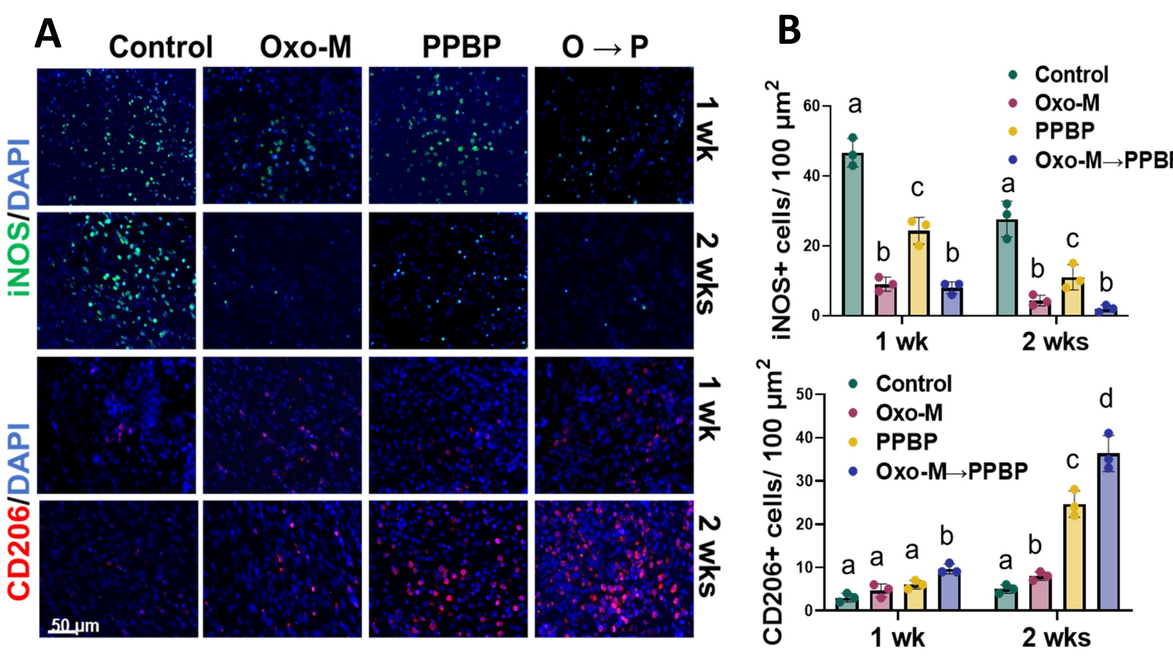


Fig. 4. Immunofluorescence show macrophage polarization (**A**). The quantitative numbers of M1 and M2 macrophages (**B**) (p<0.01; different letters indicate significant differences; n = 10 per group).

DISCUSSION

The optimized LbL fabrication process displayed an early release of Oxo-M, reducing M1-mediated inflammation, and a prolonged release of 4-PPBP, promoting M2-regulated tissue remodeling. Also, the timely controlled release of SM from the LbL PCL patch showed its efficacy in orchestrating the tissue healing processes involving inflammation, matrix synthesis, and remodeling phases.

CONCLUSIONS

Our findings indicate that the LbL nanocoated PCL patch is a promising delivery system for Oxo-M and 4-PPBP. The patch is suitable for custom-designing the release pattern and duration depending on the type, severity, anatomic location, and other complications of soft tissue injuries, including tendons and temporomandibular joint (TMJ) discs.

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