**Mapping the intrinsic immunomodulatory profiles of polymer microneedle arrays**

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**Introduction:** Biomaterials are revolutionizing vaccines and immunotherapy[1]. One exciting area is microneedle (MN) delivery[2,3]. MNs are arrays of micrometer-sized needles that efficiently deliver cargo to the specialized immunological niche of the skin. MNs are too short to reach pain receptors and do not require refrigeration, enabling global distribution. As MN use increases, there is increased need to understand the immune-modulatory properties of the polymers used for fabrication. Choosing appropriate substrates for MNs is vital because synthetic materials exhibit intrinsic properties that can bias immune responses toward either pro-immune or inhibitory effects. Here the mechanical and immune-modulatory properties of MN formed from 12 common polymeric substrates were characterized. This study provides design insight into selecting degradable polymers for MN synthesis, depending on the target immunological application.

**Methods:** MNs were fabricated using a solvent casting method of 6 different polymers, each with high and low molecular weight (M.W.) forms. Polymers were poured into negative polydimethylsiloxane (PDMS) molds, centrifuged to fill the tines, dried, and then the MNs were released from the molds. Scanning electron microscopy (SEM) was used to visualize MN integrity. Mechanical properties – including fracture force and stiffness - were measured by dynamic mechanical analysis (DMA). Immune profiling was carried out using flow cytometry to measure common activation markers of primary Dendritic Cell (DC) cultured with the MNs.

**Results:** We assessed polymers with 3 different origins: 1) those derived from extracellular matrix - gelatin and hyaluronic acid (HA), 2) naturally-derived polymers - carboxymethyl cellulose (CMC) and dextran, and 3) synthetic polymers - polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP). SEM revealed that all polymers resulted in well-formed MNs **(Fig. 1A)**. Fracture force and stiffness studies revealed dramatic differences in the properties of MNs formed from this library, both as a function of polymer structure, and in some cases, M.W. (e.g., CMC) (**Fig. 1B, 1C**). Notably, gelatin (low M.W.) and PVP MNs had fracture forces < 4N **(Fig. 1B)**; this is the minimum force needed to penetrate skin for these geometries[4], suggesting these designs might fracture before penetration. DC studies revealed a modest but significant increase in CD86 relative to PBS control for all MNs designs **(Fig. 1D)**. MNs did not significantly increase CD80 **(Fig. 1E)**. For both markers, the LPS positive control was much higher than all MN results. This suggests that even at low doses (1ng), the high M.W. polymer substrates lead to modest upregulation of CD86. Thus the polymers have some immune-activating properties, but less than LPS.

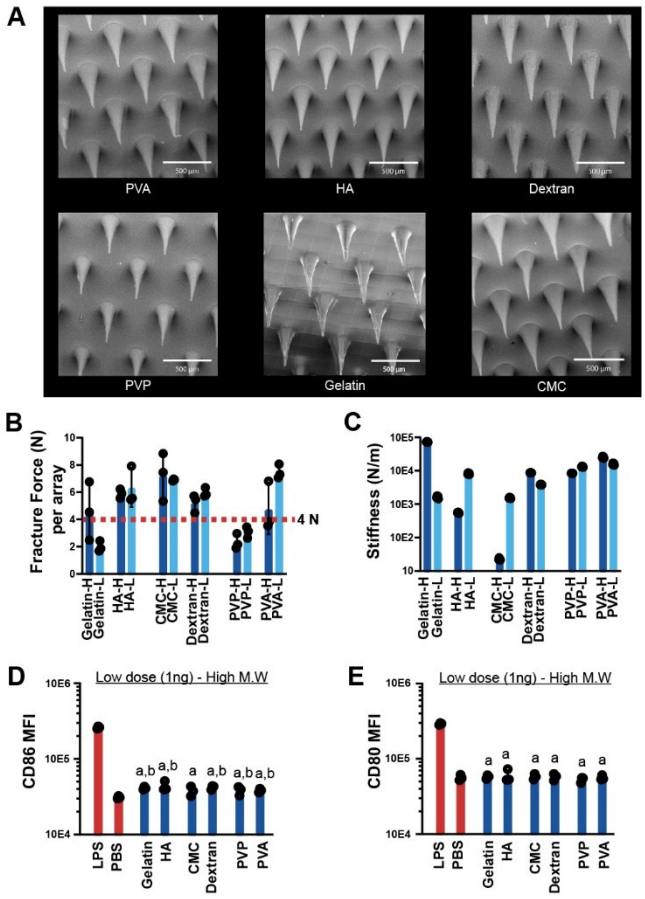


**Fig 1. | Polymer MNs lead to modest upregulation of some DC activation markers and have drastic differences in fracture force and stiffness.** A) SEM images of MNs (High M.W. polymers). B) Fracture force (N) of MN arrays. C) Stiffness (N/m) of MN arrays. D) CD86 MFI in DCs treated with high M.W. polymers respectively E) CD80 MFI in DCs treated with high M.W. polymers respectively. (a and b represent significant statistical differences ($p < 0.05$) when compared with LPS and PBS group respectively).

**Conclusion:** These studies reveal that common polymer MN substrates differ in mechanical characteristics but do not have vastly different immunomodulatory properties. These data sets could help inform the selection of MN substrates for different applications based on a combination of mechanical and immunological requirements or goals.

**References:**

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