Cell–Microsphere Interactions Mediate the Biostimulatory Effects of Calcium Hydroxylapatite and Polydioxanone Microspheres on ECM Production

Dimitrova Y[1], Hartmann C[1], Riegel K[1], Kunzler C[1], Schäfer C[1], Wollenburg C[1], Hengl T[1], Corduff N[2], Newland K.[3*]

1. Merz Aesthetics GmbH, Frankfurt, Germany. 2. Consultant in Aesthetic Medicine (Retired Specialist Plastic Surgeon), Sydney, Australia. 3. Merz Asia Pacific Pte. Ltd, Singapore. * Presenting Author.

INTRODUCTION

Calcium Hydroxylapatite (CaHA) and Polydioxanone (PDO) containing-fillers are widely utilized in aesthetic and regenerative medicine for their biostimulatory effects. Beyond their immediate volumizing or structural effects, both materials are described to stimulate ECM proteins and improve tissue quality by triggering fibroblast activity. CaHA, a mineral-like compound similar to natural bone, acts as a long-lasting natural scaffold while PDO, a biodegradable polymer, initiates a controlled inflammatory response for extracellular matrix production.

In this study, we compare the morphology of CaHA and PDO microspheres, assess their capacity for direct interaction with dermal cells, and evaluate their effects on ECM stimulation *in vitro*.

METHODS

CaHA (Calcium Hydroxapatite, Radiesse®, Merz North America, Inc., Franksville, WI, USA) and PDO (Polydioxanone, ULTRACOL®, Ultra V Co., Ltd., Seoul, Republic of Korea) microspheres were isolated and co-cultured with primary normal human fibroblasts (NHDFs) for 7 days. CaHA and PDO based microspheres were visualized by scanning electron microscopy (SEM) and particle size distribution was analyzed using dynamic light scattering (DLS). Cell-microsphere interactions were analyzed by SEM and quantified by counting the number of microspheres in contact with cells relative to the total number of microspheres expressed as a percentage. ECM stimulation by both CaHA and PDO microspheres was assessed by indirect immunofluorescence (IF) using specific antibodies targeting collagen I, and fibronectin. Pro-collagen and tropoelastin secretion were analyzed by ELISA. To enable a comparative analysis between PDO and CaHA, different concentrations of PDO microspheres were prepared according to two reference conditions: (1) equal in vitro particle concentration (1 mg/mL for both), (2) equal particle numbers (CaHA 3 mg/mL; PDO ≈0.2 mg/mL). Ordinary one-way ANOVA with multiple comparisons test (* $p<0.05$; *** $p<0.005$; **** $p<0.0001$).

RESULTS - CaHA spheres are uniform in size and undergo direct cell interaction

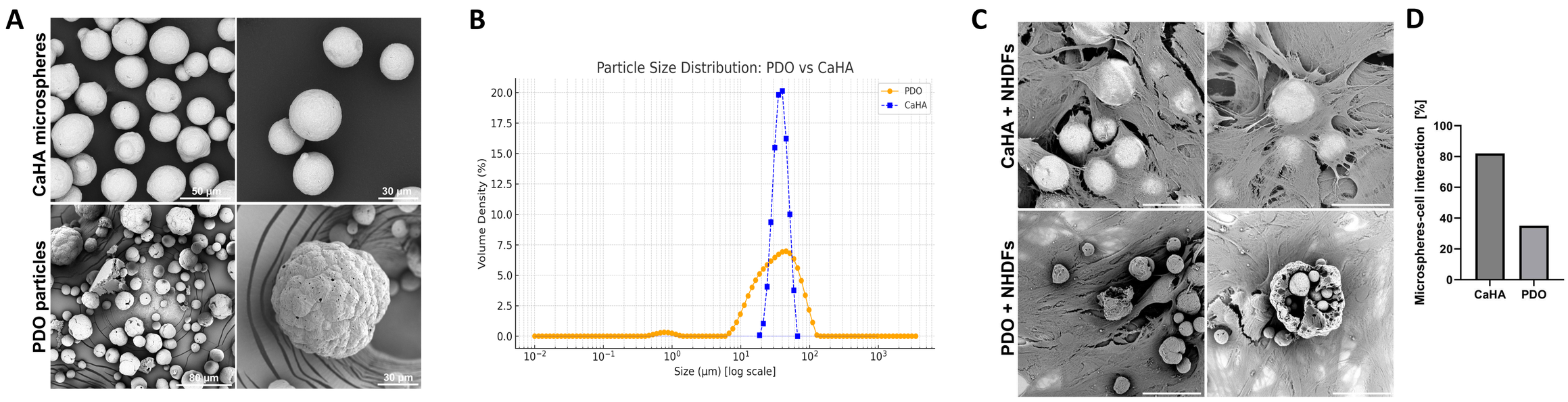

Figure 1: Differences in morphology and microspheres to cell-interaction. (A) SEM images of isolated CaHA microspheres and PDO particles. (B) Quantification of particle size distribution by dynamic light scattering (DLS). (C-D) Microsphere-cell interactions are shown for CaHA and PDO with Quantification in percentage (%).

- **CaHA microspheres are uniform in size and spherical shaped. PDO particles have a broader size range starting from 400 nm to 120 µm including particle debris.**
- **82% of CaHA microspheres undergo a direct sphere-cell interaction as visualized by the ECM network around the microspheres. For PDO particles, this interaction is reduced to 35%.**

RESULTS - CaHA provides higher biostimulatory effects compared to PDO

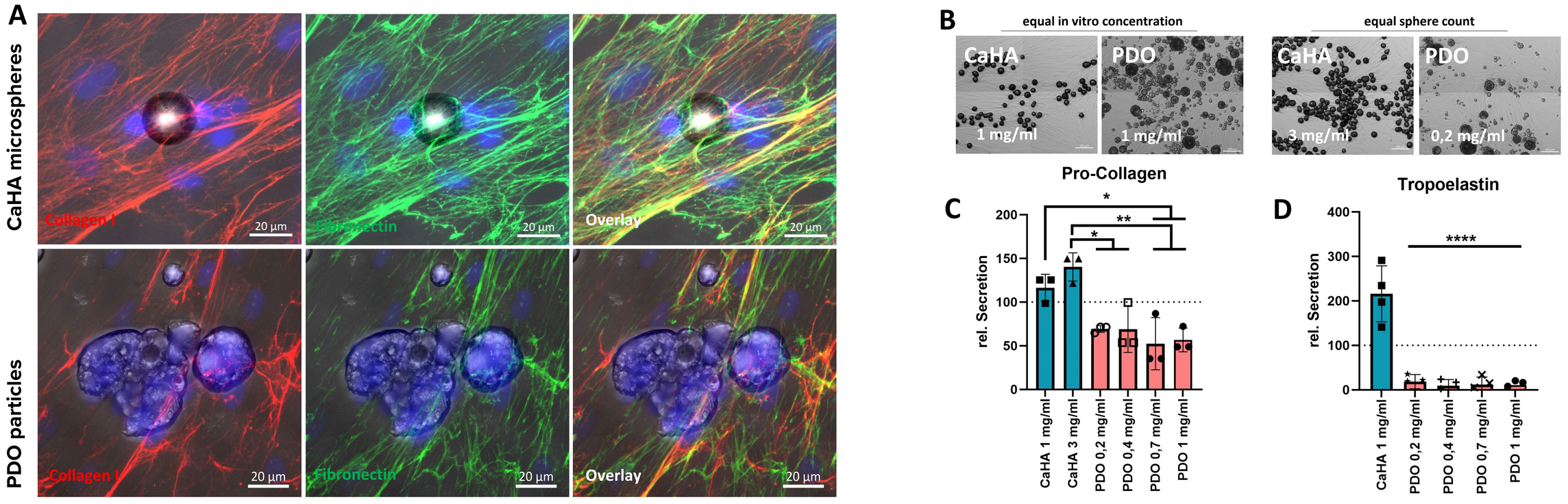

Figure 2. Collagen and ECM stimulation in fibroblasts by CaHA microspheres and PDO particles. (A) Collagen I and fibronectin expression at day 7 post-treatment with CaHA or PDO spheres visualized by immunofluorescence. (B) Exemplary bright field images of different in vitro particle concentrations. (C) Pro-collagen and tropoelastin levels measured by ELISA in response to CaHA and PDO.

- **Pronounced collagen I signal and well-organized ECM formation surrounding CaHA microspheres.**
- **CaHA microspheres induce significantly higher pro-collagen and tropoelastin secretion compared to PDO.**

CONCLUSION

- **CaHA microspheres showed greater cell-microsphere interaction and robust stimulation of ECM synthesis with significantly higher secretion of pro-collagen and tropoelastin.**
- **PDO particles exhibited markedly greater particle size heterogeneity, minimal cellular contact, and modest ECM upregulation, highlighting fundamental differences in their bioactive behavior.**

COI Disclosure: Dimitrova Y, Hartmann C, Riegel K, Kunzler C, Schäfer C, Wollenburg C, Hengl T, and Kathlea N are employees of Merz Aesthetics. Corduff N serves as a clinical advisor and lecturer for Merz Aesthetics.