Microparticulate Formulations of Antioxidant Poly(β-Amino Ester) Polymers for Wound Healing Applications

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Statement of Purpose: Oxidative Stress, (excessive production of reactive oxygen species and/or loss of antioxidant (AOX) capacity) can damage DNA, cells and tissues, and has been implicated as a disease mechanism in many acute and chronic dermal pathologies such as oral mucositis, diabetic ulcers, and severe burns. A potent AOX such as curcumin represents an exciting therapeutic agent in the treatment of non-healing dermal wounds. However, the use of AOX remains a challenge due to their poor chemical stability leading to poor shelf life of drug formulations and poor efficacy upon delivery. We have previously reported a tunable platform technology that allows conversion of polyphenolic AOX into degradable polymers via simple covalent Michael addition reactions between acrylate and amine groups (Wattamwar PP. Acta Biomaterialia. 2012;8:2529-2537). AOX released from these poly(beta-amino ester) (PBAE) polymers via hydrolytic degradation retain their activity, and protect cells from oxidative damage. Here, we present the development of microparticulate curcumin-based PBAE (C-PBAE) formulations that allow delivery of active curcumin, facilitating their application and action on wound beds.

Methods: Curcumin (Chemimpex) was first acrylated with acryloyl chloride (Sigma). Purified curcumin diacrylate (CDA) was then reacted with 4,7,10-Trioxa-1,13-tridecanediamine (TTD, Sigma, primary diamine crosslinker) and poly(ethylene glycol) diacrylate (PEGDA, MW 400, Polysciences, co-monomer) to obtain covalently crosslinked C-PBAE gels. The molar ratio of CDA and PEGDA was 1.0, and that of total acrylates to total amine protons was 1.2. C-PBAE gels were chopped into small pieces and washed in anhydrous acetonitrile for 1 h three times. Washed gel pieces were ground into fine powders using a cryogenic mill (SPEX SamplePrep Freezer/Mill 6770) in the presence of 0 or 10 % w/w magnesium stearate (MS) as dry lubricant. Particle size was measured with an UV laser-based optical size analyzer (Shimadzu SALD-7101). C-PBAE powders were degraded in PBS with 5% v/v DMSO (to ensure solubility of released curcumin) and the released curcumin was quantified at 0, 2, 4, 6, 8, 10, 12, and 24 h using spectrophotometry and verified by HPLC (Shimadzu Prominence). C-PBAE powder (10% MS) was applied as is to mouse dermal tissue, incubated in PBS at 37°C for 3 h, and imaged.

Results: Cryogenic grinding was chosen to prevent softening and degradation of the C-PBAE gel due to heat generation. In order to eliminate the presence of an additional material, we initially ground the C-PBAE gels without MS. MS free powders degraded over 24 h (blue diamonds in Fig. 1) with a calculated half-life at approximately 9.9 h. They gave an average particle size of 212±2 µm, which could be attributed to cohesion between the particles in the absence of a dry lubricant to keep them apart. This cohesion (clumping) is undesirable

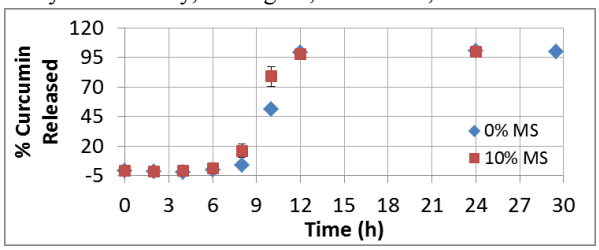

Fig. 1. Time dependent release of curcumin from C-PBAE powders ground with 0% and 10% MS.

because it is unpredictable, and the larger particle size will cause poorer surface coverage and mechanical irritation when applied to the wound bed. C-PBAE powders with 10% MS gave an average particle size of 62±1 µm. Interestingly, their release profile was identical to that without MS (red squares in Figure 1) with a half-life of approximately 8.8 h. HPLC revealed that the degradation products of both powders showed peaks corresponding to curcumin (~ 14 min in Fig. 2),

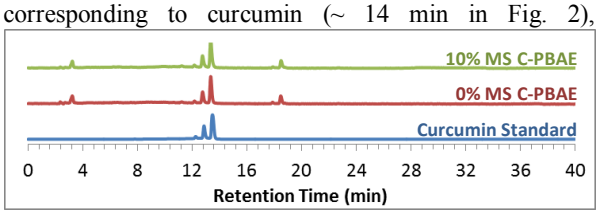

Fig. 2. HPLC of C-PBAE degradation products confirms release of curcumin.

confirming its release. As proof of concept, we chose to apply the C-PBAE powder with 10% MS to mouse dermal tissue (Fig. 3A) as the smaller particle size would provide better surface coverage and a lower the chance of mechanical irritation. The dry powder adhered to the tissue surface without any carrier material or vehicle (Fig. 3B). Under idealized experimental sink conditions of incubating the tissue coated with 10% MS C-PBAE powder in PBS at 37°C, the powder degraded slowly and a lesser amount was still visible on the tissue after 3 h (Fig. 3C).

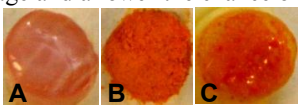

Fig. 3. C-PBAE (10% MS) powder adheres to mouse dermal tissue (A), without vehicle (B), and is visible even after 3 h of incubation in PBS at 37°C (C).

Conclusions: The antioxidant curcumin can be readily converted into degradable polymers, and formulated as fine powders using a simple grinding technique. C-PBAE powders without and with 10% MS provide very similar delayed release profiles and half-lives. The release of curcumin was confirmed by comparative HPLC analysis. We have also demonstrated that dry C-PBAE powder can be directly applied to dermal tissues without any carrier material. Due to the obvious advantages presented by powders synthesized with a dry lubricant and having smaller particle size, the 10% MS C-PBAE powder formulation has become the candidate for more thorough investigations of their physical stability, and the activity of curcumin released on dermal tissues.