Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary figures

A collagen-AS/εPLL bilayered artificial substitute regulate anti-inflammation and infection for initial inflamed wound healing

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Supporting Figures

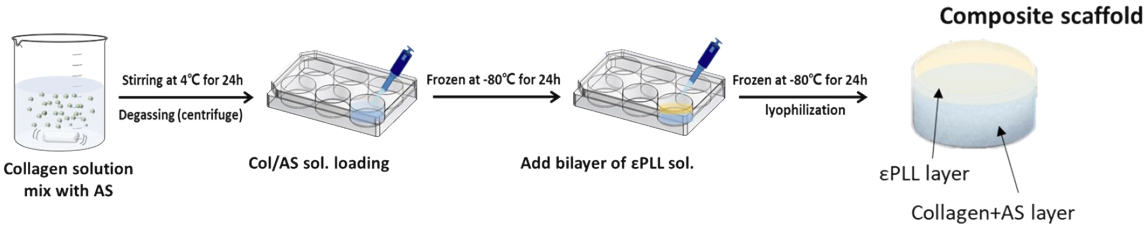


Fig. S1. Scheme of the preparation of bilayerd composite scaffold. The three-step fabrication process includes: After homogenizing AS to the collagen solution, it loaed on the well plate. And after deep freezing then εPLL is loaded as a bilayer on the freezed collagen-AS. Lastly deep freezing followed by lyophilization to obtain a bilayered composite scaffold.

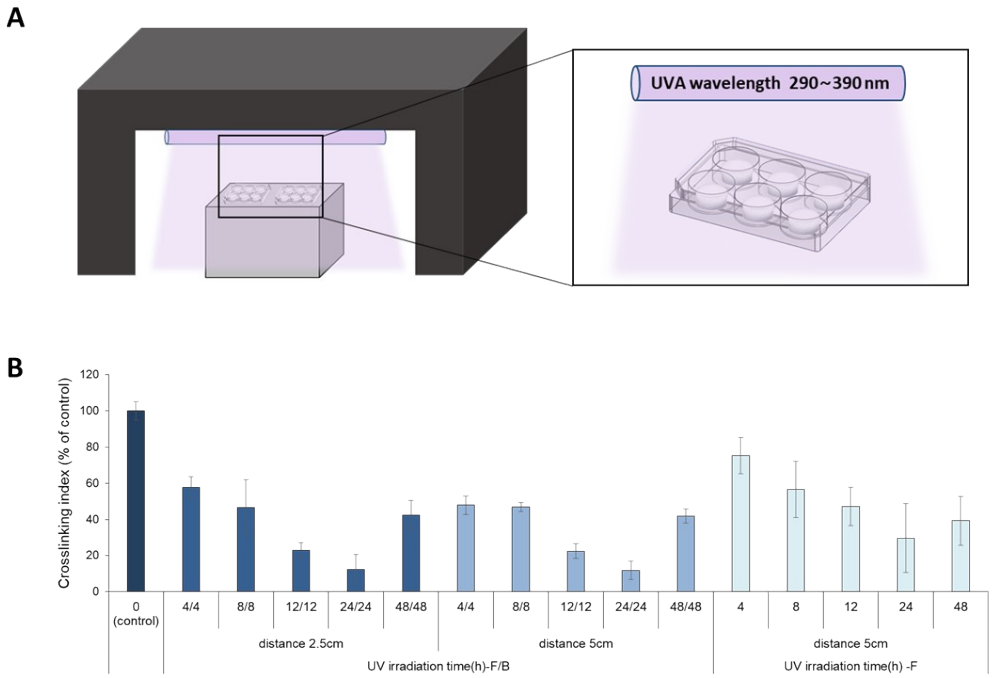


Fig. S2. The composite scaffold were subjected to UV irradiation below a halogen UV lamp (wavelength range 290–390 nm). (A) Scheme of the preparation of irradiation method on composite scaffold. The light intensity at this distance was determined as 65mW/cm^2 by a UV radiometer. (B) The graph of screening to determine the most effective irradiation value.

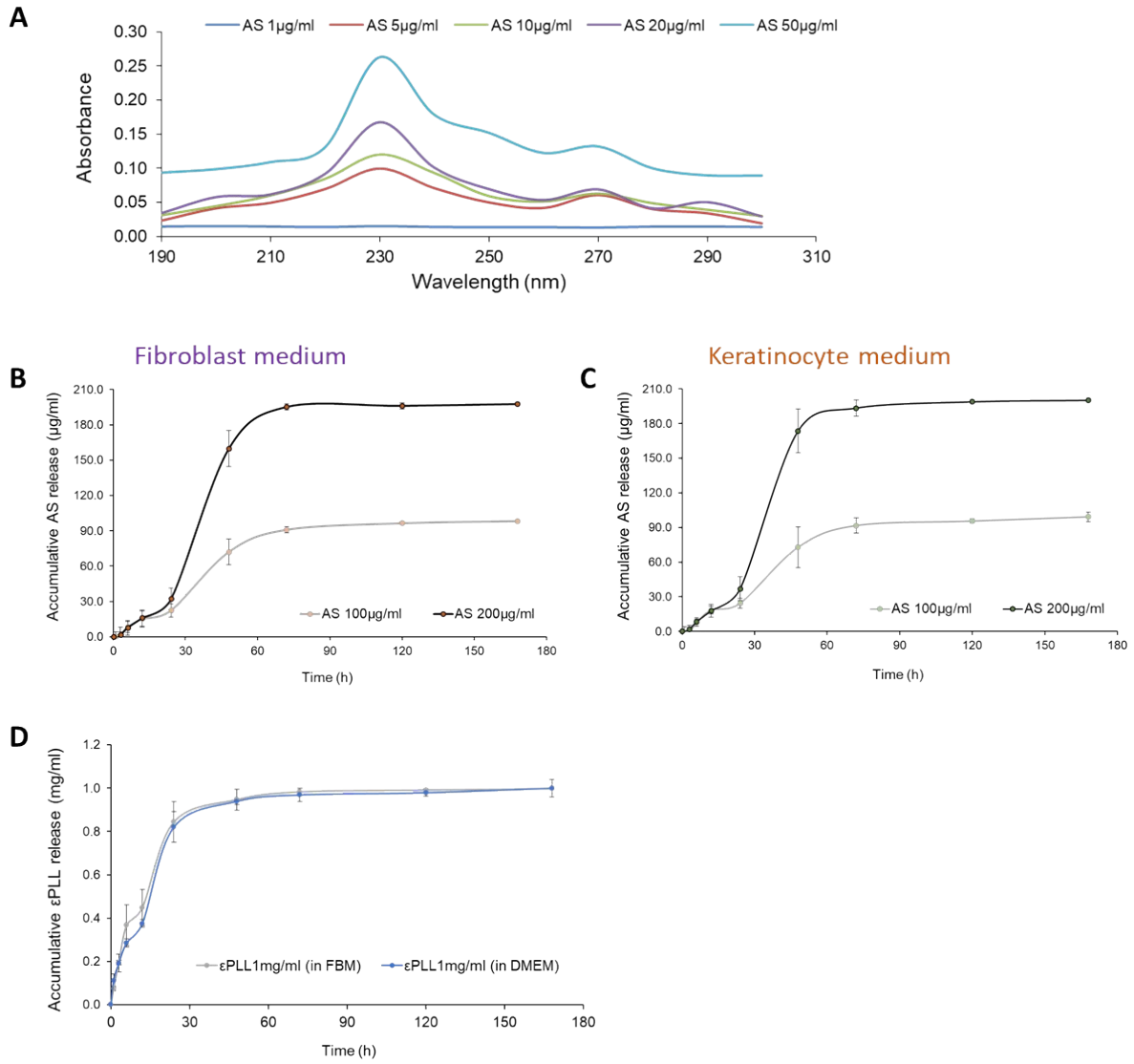


Fig. S3. *In vitro* accumulative release profile of asiaticoside from the Collagen-AS/εPLL composite scaffold. (A) Representative of wavelength screening in different concentration of AS were 1, 5, 10, 20 and 50 µg/ml. A graph measured the amount of release accordingly to the AS concentration of 100 and 200 µg/ml in (B) fibroblast medium (FBM) and (C) keratinoncyte medium (DMEM). D) Accumulative εPLL release in FBM and DMEM medium. Error bars represents mean ± standard deviation (n = 4).

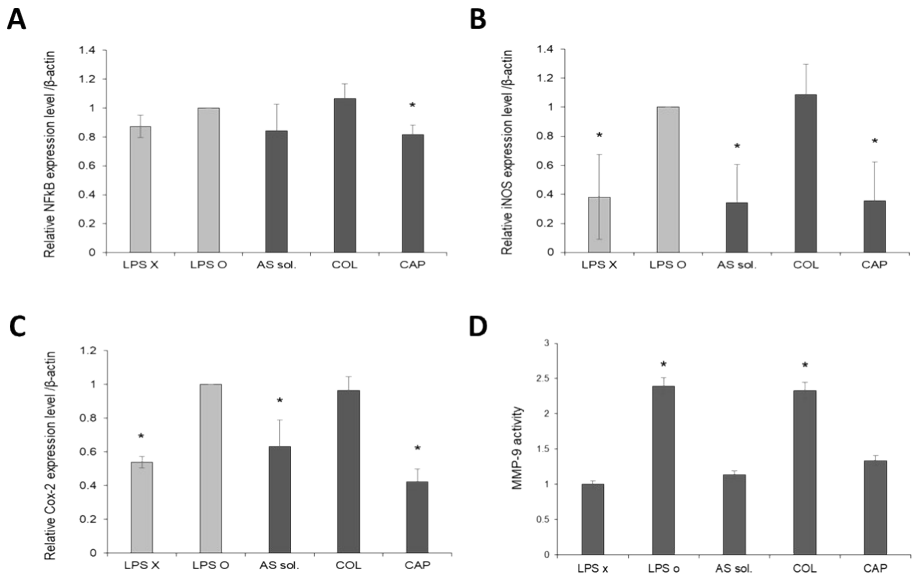
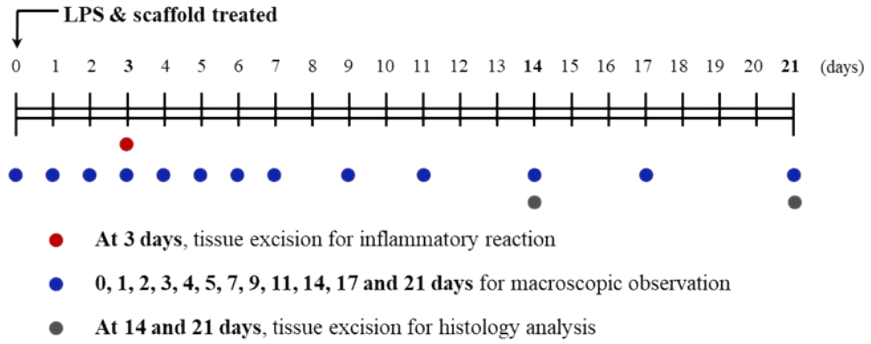


Fig. S4. The graphs indicated protein expression levels (westernblot) of (A) NF-kB, (B) iNOS, (C) Cox-2 and (D) MMP-9 in macrophage. The amount of protein level measured using Image J. Data are expressed as means ± SD, *$p < 0.05$ vs. only LPS treated cell (LPS o).

A

Group 1	**Sham (untreated)** : only wound
Group 2	**Control** (w/ LPS) : LPS treatment on the wound
Group 3	**COL** (w/ LPS) : LPS and collagen scaffold treatment on the wound
Group 4	**CAP** (w/ LPS) : LPS and collagen-AS/εPLL scaffold treatment on the wound

B



C

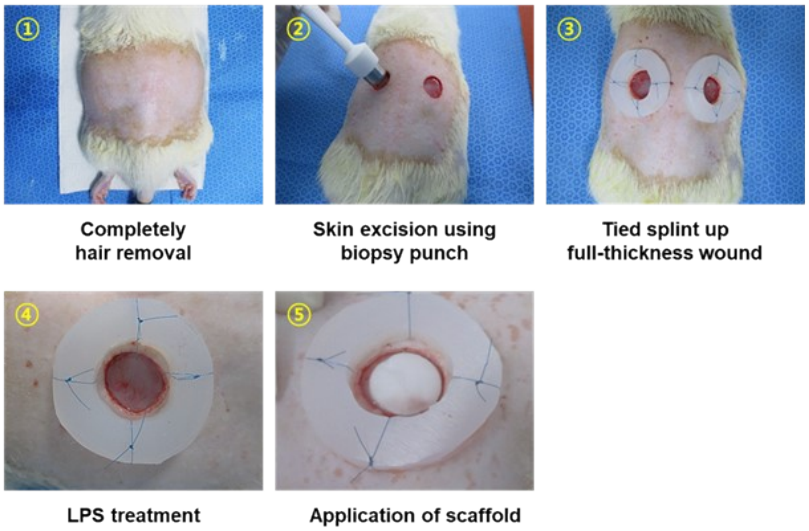


Fig. S5. (A) *in vivo* experimental groups and final study design: optimized inflamed full-thickness wound model. For in vivo study, healthy male SD rats were randomly assigned to experimental groups and they were divided into 4 groups untreated, sham with only wounds, Control group with LPS treatment, COL group is LPS with collagen scaffold treatment, and CAP

group is collagen with AS/εPLL scaffold treatment. (B) all groups were excision at day 3 to measure the initial inflammatory response and histological analysis. And from day 0 to 21, macroscopic observation to measure wound size and weights. Lastly confrimed wound healing effect on second and third weeks by isolated wound skin for histology analysis. (C) method of LPS induced inflammation dorsum wound model of SD rat.

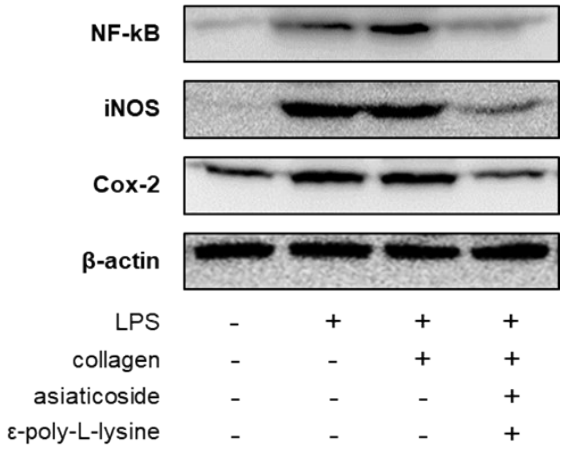


Fig. S6. *In vivo* inflammation test by quantification of inflammation related protein levels were examined (at 3 days) by western blot in skin wound section.