Electronic Supplementary Material (ESI) for RSC Advances.


# Supporting Information

## Development of Biodegradable Zein-Based Bilayer Coatings for Drug-Eluting Stents

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**Table S1**. Characteristic FTIR absorption bands assigned to vibrational modes of zein, rutin and rutin-loaded zein functional groups.

| FT-IR peaks ($cm^{-1}$) | | | Vibrational mode |
|---|---|---|---|
| Pure Zein | Pure Rutin | Rutin-loaded Zein Film or ZR | |
| 3229 | 3327 | 3283 | Amide A (ν(O-H), ν(N-H)) |
| 2959-2876 | 2986-2874 | 2961-2882 | ν(C-H) |
| 1641 | 1653 | 1641 | Amide I (ν(C=O)), ν(Ar-C=O) |
| 1533 | | 1533 | Amide II (ν(C-N), δ(N-H)) |
| 1449 | 1454 | 1443 | δ(C-H), ν(C=C aromatic ring) |
| | 1360 | 1360 | δ(C-OH) |
| 1302-1240 | 1296-1271 | 1306-1242 | Amide III (δ(N-H), ν(C-N)), ν(C-C-O) |
| | 1202 | 1209 | ν(C-O-C) |
| 1169 | | 1171 | ν(C-N) |
| 1124 | 1132-1123 | 1124 | ν(C-OH), ν(C-O-C) |
| | 1013-999 | 1015 | ν(C-O) |
| | 943 | 947 | ν(C-C) |

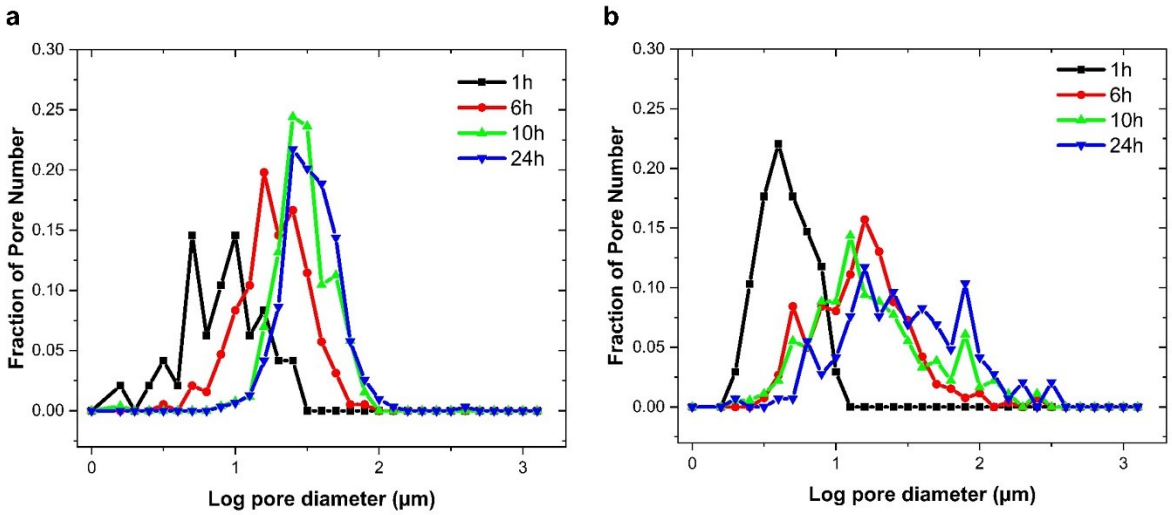


**Table S2**

| | Zein | | Rutin-loaded Zein | |
|---|---|---|---|---|
| | Mean pore diameter (μm) | Median pore diameter (μm) | Mean pore diameter (μm) | Median pore diameter (μm) |
| 1h | 0.866 | 0.757 | 0.479 | 0.429 |
| 6h | 1.891 | 1.674 | 2.130 | 1.584 |
| 10h | 2.883 | 2.554 | 2.985 | 1.587 |
| 24h | 3.341 | 3.012 | 4.748 | 2.755 |

**Figure S1. Pore size distribution.** The pore size distribution was quantified from the SEM images of zein (a) and ZR (b) coated samples, using ImageJ software. On average, 200 pores from 3 different SEM micrographs were analyzed for each time point. The values of the mean and median diameters of the pores can be consulted in Table S2.

**Table S3**. Characteristic FTIR absorption bands assigned to vibrational modes of cross-linked alginate, ZR and ZR/cAlg functional groups.

| FT-IR peaks ($cm^{-1}$) | | | Vibrational mode |
|---|---|---|---|
| cAlg | ZR | ZR/cAlg | |
| 3227 | 3283 | 3373 | Amide A (ν(O-H), ν(N-H)) |
| 2924 | 2961-2882 | 2955 | ν(C-H) |
| 1589 | 1641 | 1636 | $\nu_{as}$(C-O-O), Amide I (ν(C=O)) |
| - | 1533 | 1518 | Amide II (ν(C-N), δ(N-H)) |
| - | 1443 | 1449 | δ(C-H), ν(C=C aromatic ring) |
| 1406 | - | 1389 | $\nu_{s}$(C-O-O) |
| - | 1360 | 1366 | δ(C-OH) |
| 1296 | 1306-1242 | 1310-1240 | Amide III (δ(N-H), ν(C-N)), ν(C-O) |
| - | 1171 | 1171 | ν(C-N) |
| 1123 | 1124 | 1130 | ν(C-OH), ν(C-O-C) |
| 1078 | - | 1065 | ν(C-O) pyranose ring |
| 1022 | 1015 | 1013 | ν(C-O-C), ν(C-C) |
| 947 | 947 | 951 | ν(C-O) |
| 885 | - | 891-839 | δ(C-H) mannuronic residues |

**Table S4**. Atomic percentages of the elements obtained via the XPS analysis of zein, rutin-loaded zein (ZR), ZR/Alg and ZR/cAlg coatings.

| Atomic Percentage (At %) | | | | | | |
|---|---|---|---|---|---|---|
| | C 1s | O 1s | N 1s | Na 1s | Ca 2p | Cl 2p |
| Zein | 73.3 | 16.1 | 10.6 | - | - | - |
| Rutin-loaded Zein (ZR) | 77.7 | 14.5 | 7.8 | - | - | - |
| ZR/Alg | 71.4 | 23.8 | 2.8 | 1.4 | 0.5 | - |
| ZR/cAlg | 53.4 | 16.3 | 2.5 | - | 9.5 | 18.3 |

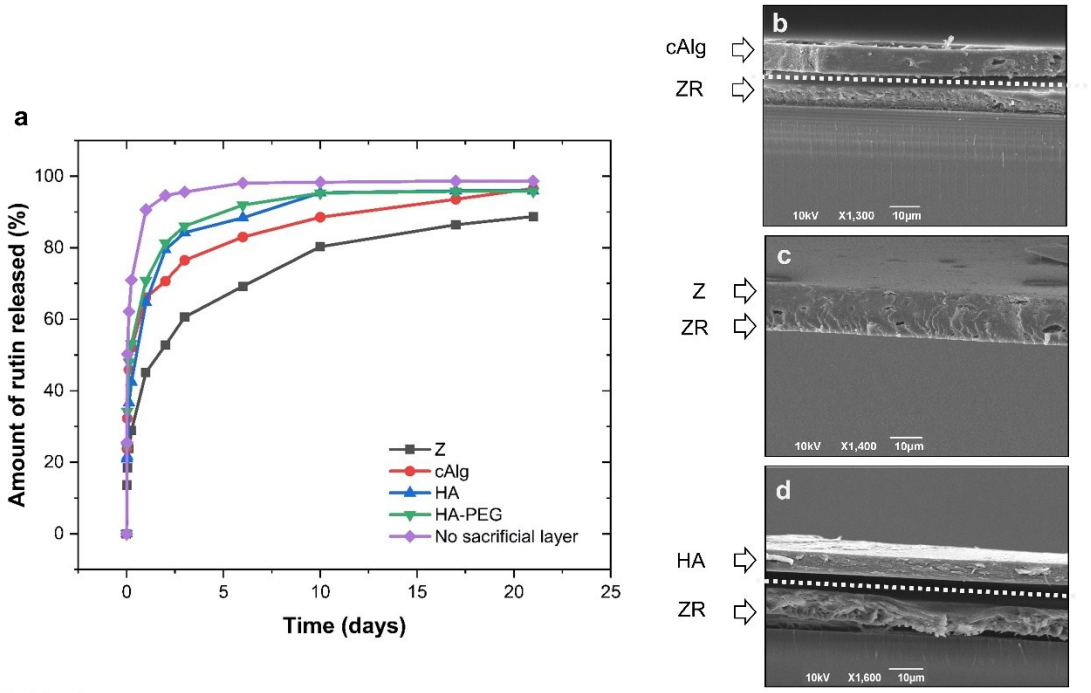


**Table S5**

| Sacrificial Layer | Average thickness (μm) | Amount of rutin released (%) |
|---|---|---|
| Z | 7.4 | 88.7 ± 4.1 |
| cAlg | 8.4 | 93.5 ± 5.9 |
| HA | 5.5 | 95.3 ± 1.0 |
| HA - PEG | 5.9 | 95.6 ± 2.7 |

**Figure S2**. **Characterization of bilayers fabricated with different sacrificial layers.** Cumulative release profile of rutin from different bilayer coatings (a): the sacrificial layers are composed of zein (Z,■), double cross-linked alginate (cAlg,●), hyaluronic acid (HA,❑) and hyaluronic acid-PEG (HA-PEG,❑). Cross-sectional view SEM images of different bilayer coatings made with cross-linked alginate (b), zein (c) and hyaluronic acid (d). Dotted lines in the SEM images delimit the separation between the two layers. Table S5 reports the average thickness and amount of rutin released for each natural polymer investigated as sacrificial layer.

The bilayers, fabricated with different natural polymers, were prepared and the drug release kinetics were determined at regular intervals for a period of 21 days. As shown in Figure S2a, compared to other polymers such as hyaluronic acid, the addition of cross-linked alginate results in a slower rutin-release rate from the underneath ZR layer coating. Moreover, alginate was chosen as sacrificial layer due to its inherent non-thrombogenic and hydrophilic nature and for its ability to control drug release when cross-linked. In the relative cross-sectional SEM image (Figure S2b), cross-linked alginate and rutin-loaded zein layers appear well-separated and clearly visible, with the sacrificial layer being well-attached to the underneath protein film.

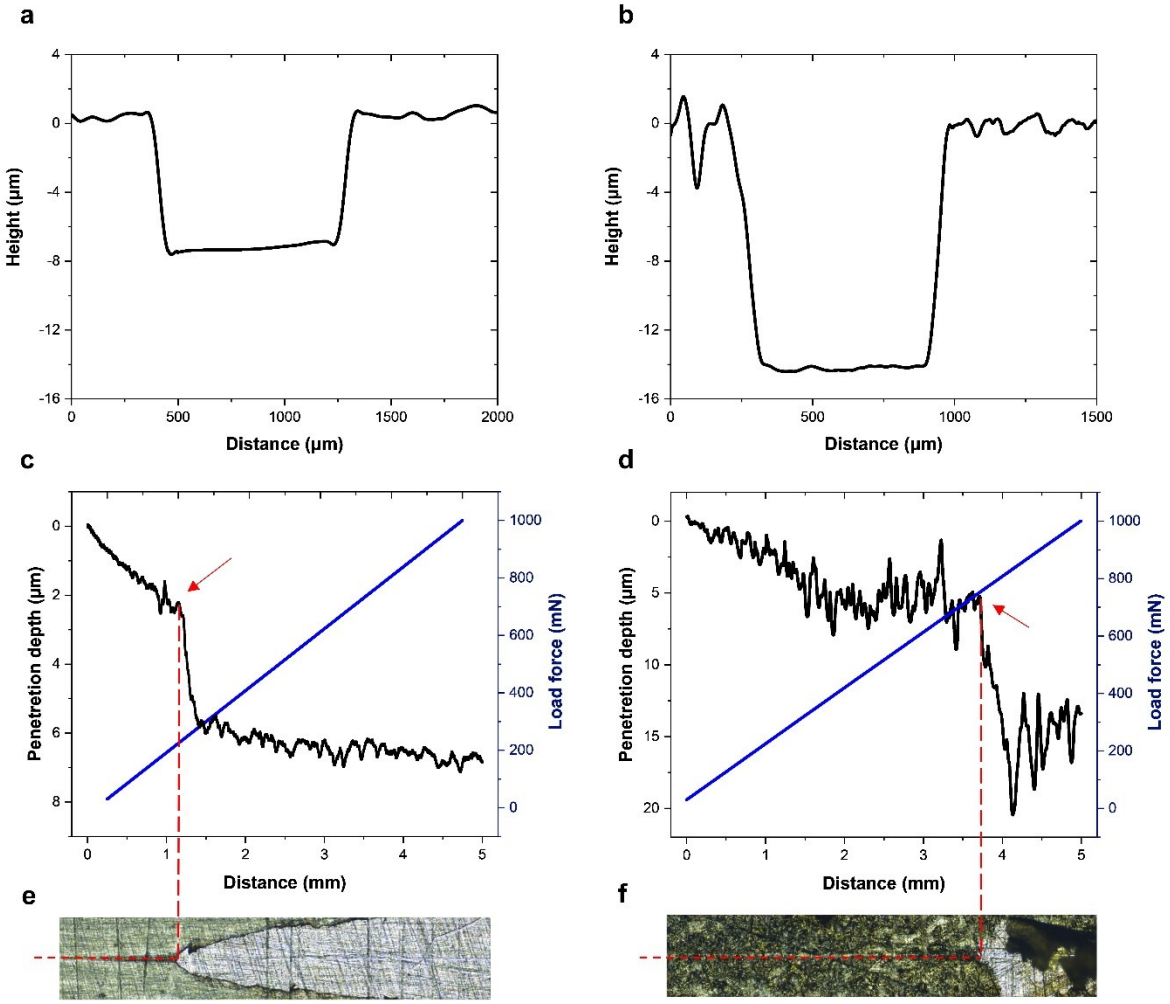


**Figure S3. Surface and mechanical characterization.** Thickness measurements of the scratched ZR (a) and ZR/cAlg (b) coated samples performed with a surface profilometer. Load-displacement curves and corresponding optical images of the ZR (c, e) and ZR/cAlg (d, f) coated specimens. Red dotted lines were drawn in order to compare the curves with the optical images of the scratch paths and to determine the exact critical load value for each coating. The red arrows indicate the drop in the penetration depth signal, which corresponds to the critical load value at the onset of coating cracking.

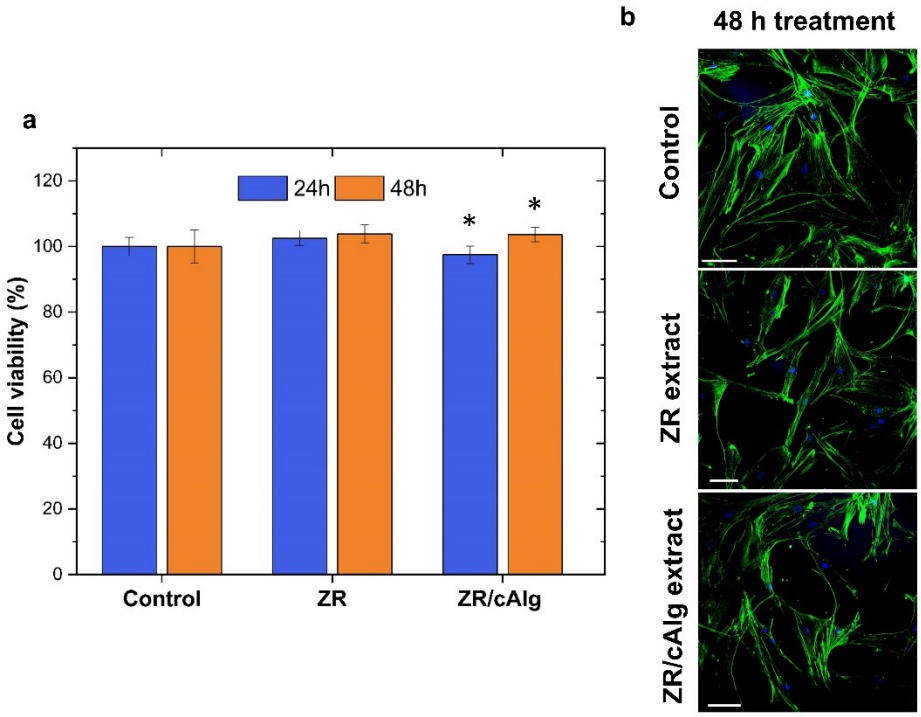


**Figure S4. Primary Human Dermal Fibroblasts biocompatibility.** On the left: HDFa cells viability (a) evaluated via MTS assay for samples treated with ZR and ZR/cAlg cell culture medium extracts and under standard conditions (control, normal growth medium), for 24 and 48 hours; data are presented as mean values ± SD. Significance (*$p < 0.05$) was observed for ZR/cAlg with respect to the control. On the right: confocal images (b) of fibroblasts exposed to ZR and ZR/cAlg coatings extracts: cell nuclei marked in blue (DAPI) and cell cytoskeletons highlighted in green (Alexa Fluor 488 Phalloidin) after 48 hours of treatment (scale bar 100 μm).

Following MTS assay, cell viabilities of 102.5 % and 97.5 % were registered after 24 hours with ZR and ZR/cAlg layers, respectively. A slightly higher cell survival is observed when HDFa cells are cultured for 48 hours, showing 103.8 % and 103.6 % cell viability with ZR and ZR/cAlg layers, respectively. As shown in Figure S4a, samples in contact with ZR/cAlg layers showed a significance (* $p < 0.05$), when compared to the control, both at 24 and 48 hours. The morphology and cytoskeletal arrangements of HDFa cells treated with different coatings extracts were detected by fluorescence staining after 48 hours and are presented in Figure S4b. After the treatments, the HDFa cells uniformly spread onto the glass substrates, indicating a healthy state.