Supplemental Data

The IR spectrum of HACC and chitosan shows evidence of the introduction of the quaternary ammonium salt group to the chitosan backbone. A peak at 1480 cm⁻¹ was assigned to the C-H bending of the trimethylammonium group (Supplemental Fig. 1). The N-H bending (1587 cm⁻¹) of the primary amine becomes weak due to the partial change of the primary amine of chitosan to the secondary amine. In addition, the HACC spectrum shows a broader band than chitosan at around 3400 cm-1 due to the increased number of hydroxyl groups [Lim, S. H., & Hudson, S. M. (2004). Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. Carbohydrate Research, 339, 313-319.].



Supplemental Fig. 1. FT-IR spectra of chitosan and HACC.

The antibacterial effects of MBG-HACC and MBG-HACC-Zein were significantly higher than that of MBG at 24h for Escherichia coli (P < 0.01, Supplemental Fig. 2 (A)), and no significant difference was observed between MBG-HACC and MBG-HACC-Zein at 24h for Escherichia coli (P > 0.05). But at 72h for Escherichia coli, the antibacterial effects of MBG-HACC-Zein were significantly higher than those of MBG and MBG-HACC (P < 0.01, Supplemental Fig. 2 (A)). Between MBG and MBG-HACC, there was no significant difference was observed at 72h for Escherichia coli (P > 0.05).



Supplemental Fig. 2 Projected top views of antibacterial efficacy of different scaffolds against Escherichia coli at 24h and 72h obtained using confocal laser scanning microscopy (CLSM) after staining with the Baclight dead/live stain. Bacteria were stained with green fluorescent SYTO 9 and red fluorescent propidium iodide, which causes live cells to appear green and dead cells to appear red under CLSM. Magnification, ×400.

HACC-Zein-modified MBG was characterized by low levels of bacteria on the surface at both 24 hours and 72 hours, which demonstrates that it possesses both short-term and long-term antibacterial activities. This is because HACC is water soluble and Zein is not. During the degradation of the scaffold, Zein can slow the release of HACC, resulting in prolonged antibacterial action. In order to further prove the result, we plotted a curve of gradual release of HACC from MBG-HACC and MBG-HACC-Zein scaffolds when they were incubated in SBF for 72 hours. The results showed that HACC almost released completely from MBG-HACC scaffolds within 12 hours after soaking, while the release of HACC from MBG-Zein-HACC scaffolds occurred significantly more slowly, with only 50.3% of HACC had released at 72 hours after soaking due to the protection of Zein coating, which would ensure prolonged antibacterial effects of MBG-HACC-Zein scaffolds (see Fig.10 C). In addition, FT-IR and TGA results of MBG-HACC scaffolds after soaking in SBF for 72 hours after soaking in SBF for 72 hours.



Supplemental Fig. 3. FT-IR spectra of MBG-HACC scaffolds after soaking in SBF for 72 h.



Supplemental Fig. 4. TGA of MBG and MBG-HACC scaffolds after soaking in SBF for 72 h.