Supplementary Information

The semi-interpenetrating network hydrogel loaded with Oridonin and DNase-I on healing of chemoradiotherapy-induced oral mucositis

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Determination of radiotherapy dosage and timing in rats

Before establishing the oral mucositis (OM) rat model post-radiotherapy, we designed five groups, each with 7 rats, with different doses and timing of radiotherapy. By observing general signs in rats post-radiotherapy, such as lethargy, reduced activity, hunching, alopecia (Figure S1), mucosal ulceration and bleeding (Figure S2), increased salivation, decreased food intake, weight loss, and mortality rates, we aimed to determine the optimal dosage and timing of radiotherapy in rats. The fifth group showed a 0% mortality rate at 3 and 7 days, while displaying clear typical symptoms of post-radiotherapy (Table S1). Therefore, the dosage and timing of radiotherapy in this group were determined as the optimal conditions for establishing the chemoradiotherapy-induced OM.

Supplementary Figure S1：Characteristics of rats with alopecia after radiotherapy
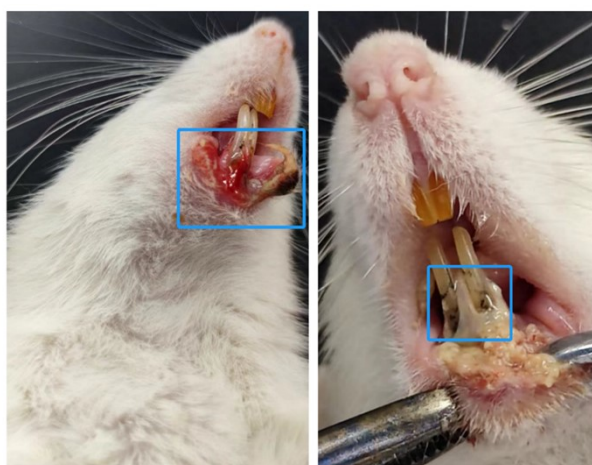
Supplementary Figure S2：Characteristics of rats with mucosal ulceration and bleeding after

radiotherapy

Table S1 Mortality rate of rats after different doses of radiotherapy

Does cGy	Time min	Total pcs/group	Typical Symptoms pcs/group	Day of Death d	Amount of Death pcs/group	Mortality Rate %
400	3.5	7	7	3	4	57.14
				7	5	71.43
300	2	7	7	3	3	42.86
				7	4	57.14
250	2.5	7	7	3	1	14.29
				7	3	42.86
200	2	7	7	3	2	28.57
				7	2	28.57
200	1	7	7	3	0	0
				7	0	0

**Effect of ORI/DNase-I/IPN on colony survival fraction detected by colony assay**

The gold standard for measuring the radiosensitivity of cells *in vitro* is the clonogenic assay. *[Radiotherapy and Oncology. 2019 Oct 1;139:87-93].* We used L929 cells to study the effect of ORI/DNase-I/IPN on colony survival fraction detected by colony assay. After seeding L929 cells into plates, they were incubated in a cell culture incubator for 24 hours to allow for adherence. Subsequently, following X-ray exposure (X-ray irradiator, X-Rad 225XL, total RT Dose 200cy, 1.5min), hydrogel or drug solutions were added to each group, and the effects on cell cloning were evaluated. As shown in Figure S3, incubation with ORI/DNase-I/IPN did not result in a significant difference in cell survival fraction compared to the control group.
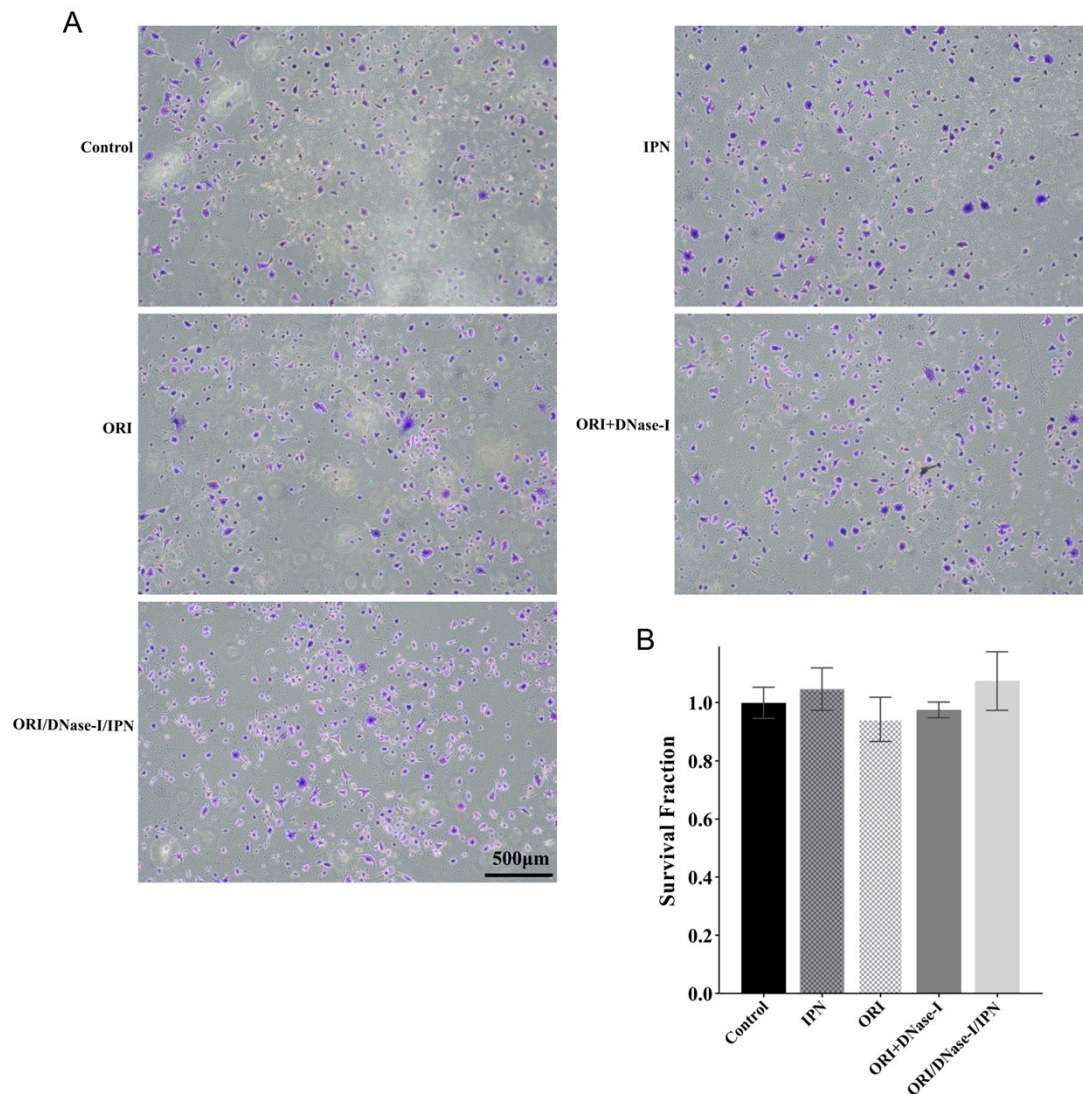
Supplementary Figure S3：(A) L929 cell survival fraction after radiotherapy (B) their statistical analysis

The degradation of NETs *in vivo*

In order to investigate the degradation effect of NETs at the wound after ORI/DNase-I/IPN treatment chemoradiotherapy-induced OM, we specially bred a group of OM model rats and gave DNase-I treatment. At 7 days (7d), wound proteins were extracted and WB experiments were performed to examine the ability of DNase-I to degrade NETs at the chemoradiotherapy-induced OM wound for comparison with ORI/DNase-I/IPN. Chemoradiotherapy-induced OM treated with DNase-I, as shown in Figure S4.
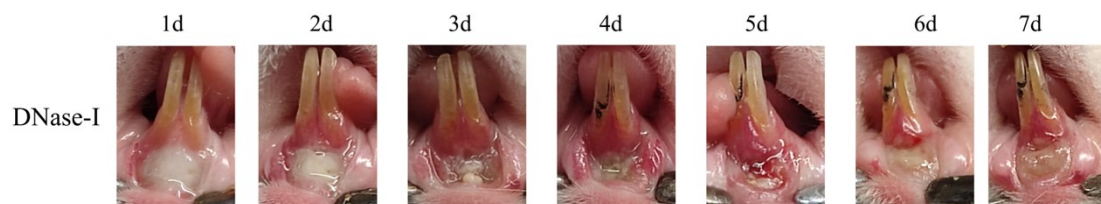
Supplementary Figure S4：Actual OM changes after 7 d of treatment with DNase-I

MALDI-TOF MS: identification bacteria strains

MALDI-TOF MS is a technique that can identify different microorganisms quickly and accurately. Detection of oral mucositis secretions following radiotherapy and chemotherapy using the MALDI-TOF MS system showed that *Staphylococcus aureus, Escherichia coli and Streptococcus oralis* were not detected in ORI/DNase-I/IPN groups (Figure S5-7).
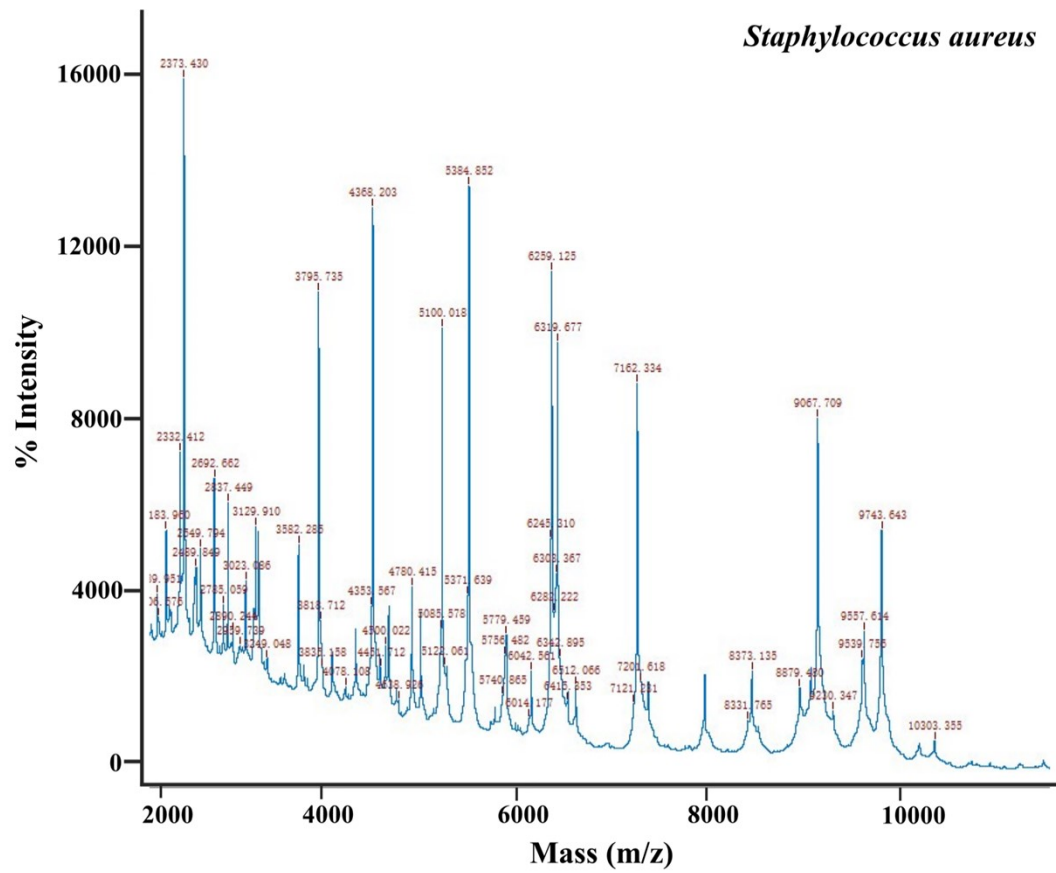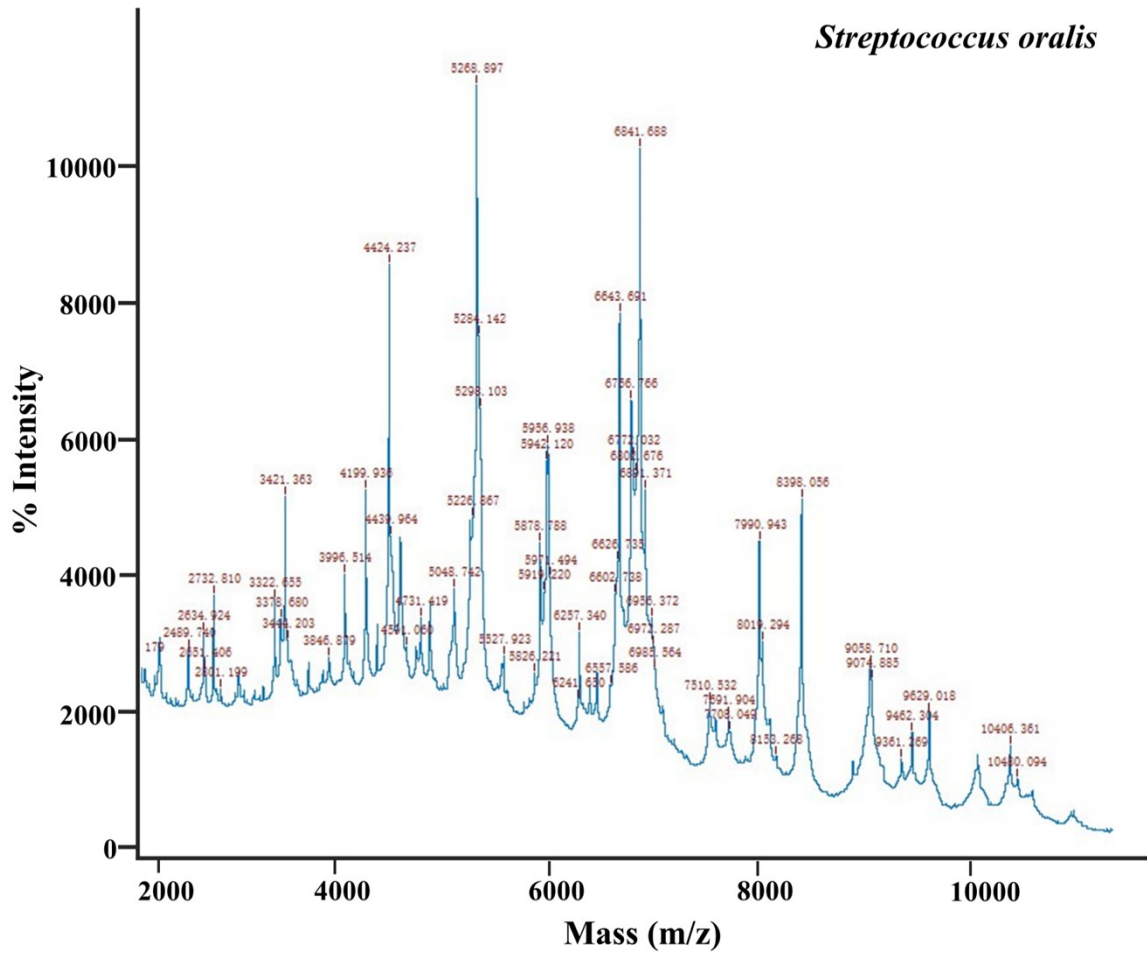
Supplementary Figure S5：MALDI-TOF MS detection of *Staphylococcus aureus*.
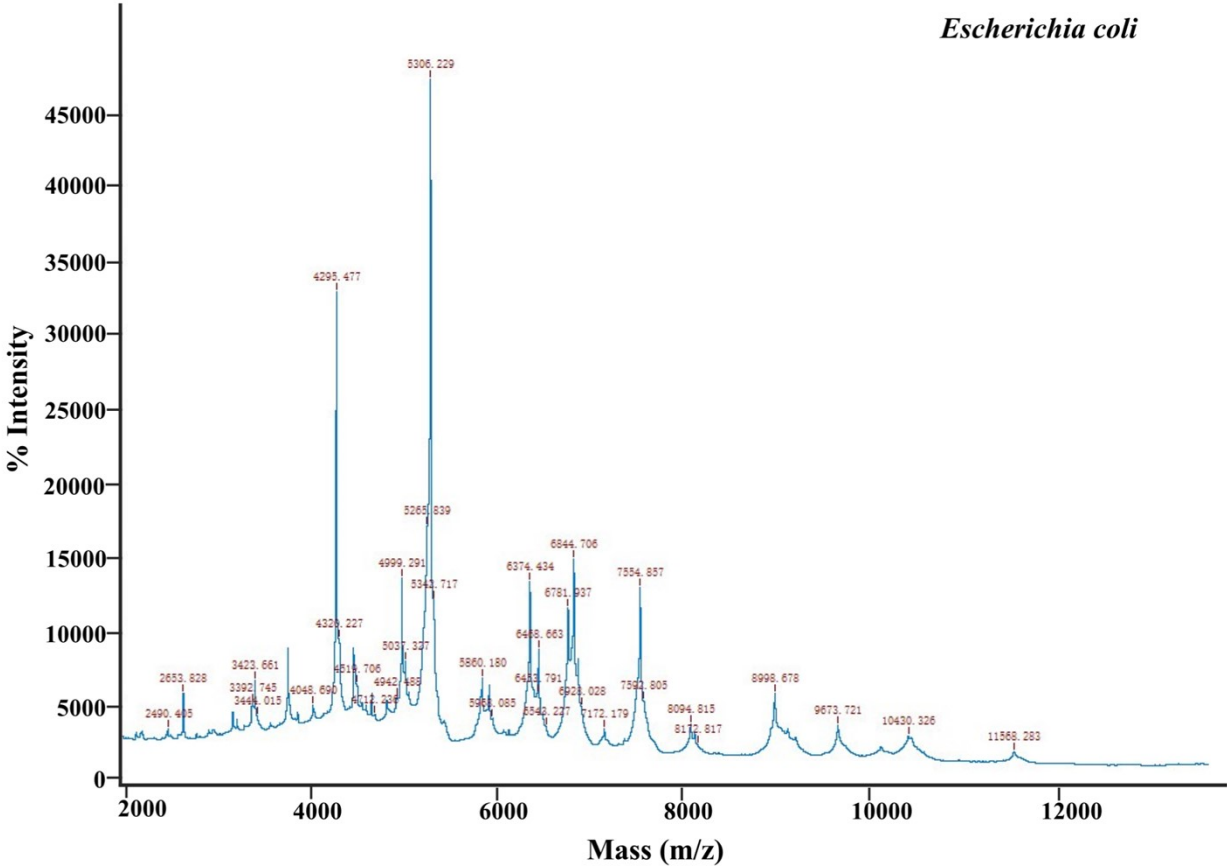
Supplementary Figure S6：MALDI-TOF MS detection of *Escherichia coli.*

Supplementary Figure S7：MALDI-TOF MS detection of *Streptococcus oralis*.