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Supplementary information for

Osteoinduction and long-term osseointegration promoted by combined effects of nitrogen and manganese elements in high nitrogen nickel-free stainless steel

Yiqiang Yu, Tingting Ding, Yang Xue, and Jiao Sun*

Shanghai Biomaterials Research & Testing Center, Shanghai Key Laboratory of Stomatology, Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200023, China.

Table S1. Primers used for real time RT-PCR.

| Gene | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') |
|---------------------|---------------------------------|---------------------------------|
| GAPDH | GGCAAGTTCAACGGCACAGT | GCCAGTAGACTCCACGACAT |
| Integrin $\alpha 1$ | AGCTGGACATAGTCATCGTC | AGTTGTCATGCGATTCTCCG |
| Integrin β1 | AATGTTTCAGTGCAGAGCC | TTGGGATGATGTCGGGAC |
| RUNX-2 | GCTTCTCCAACCCACGAATG | GAACTGATAGGACGCTGACG |
| | | A |
| ALP | CGGAAGTGAGGCAGGTAG | AGAGCCCACAATGGACAG |
| OPN | TGGATGAACCAAGCGTGGA | TCGCCTGACTGTCGATAGCA |
| OCN | GTGCCGTCCATACTTTCG | GACCACATTGGCTTCCAG |

^{*} Corresponding address: jiaosun59@126.com

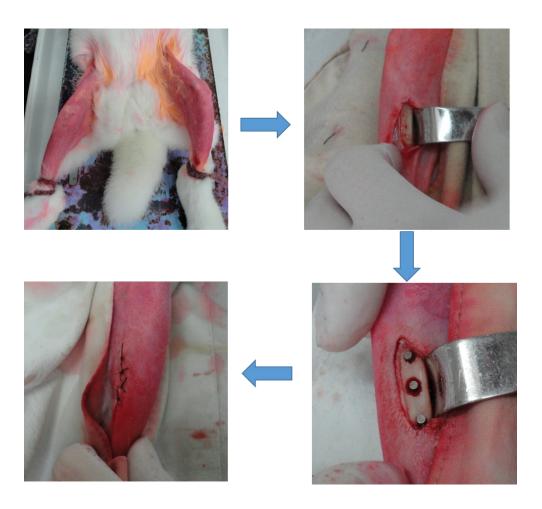


Figure S1. Surgical implantation procedure:

Rabbits were injected intramuscularly with ketamine at a dose of 0.5 mg/kg. After the midshaft of tibia was exposed, three holes (Φ 2.2 mm, 10 mm apart) were prepared perpendicularly at the specific site. Three implants of each material (HNS and 316L SS) were inserted into left and right tibiae respectively and then the surgical wounds were sutured closely.

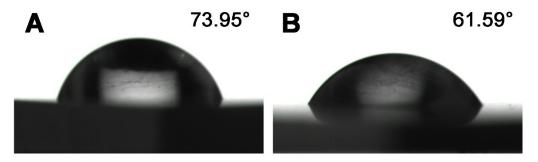


Figure S2. Water contact angle images of (A) 316L SS and (B) HNS.

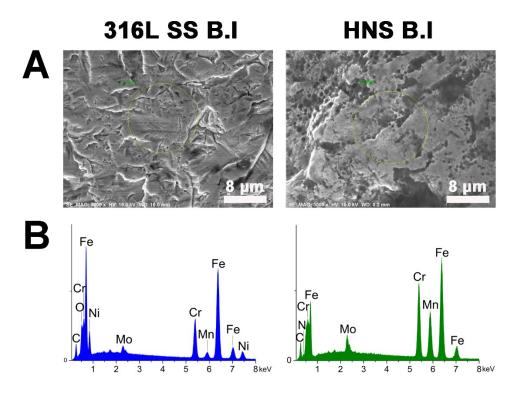


Figure S3. Morphology and element composition of 316L SS and HNS surfaces before implantation (B.I). (A) SEM top-view of surface microstructures; (B) EDS spectra of the circular region in corresponding SEM images.