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SUPPLEMENTARY INFORMATION

Localized drug delivery of selenium (Se) using nanoporous anodic aluminium oxide for bone implants

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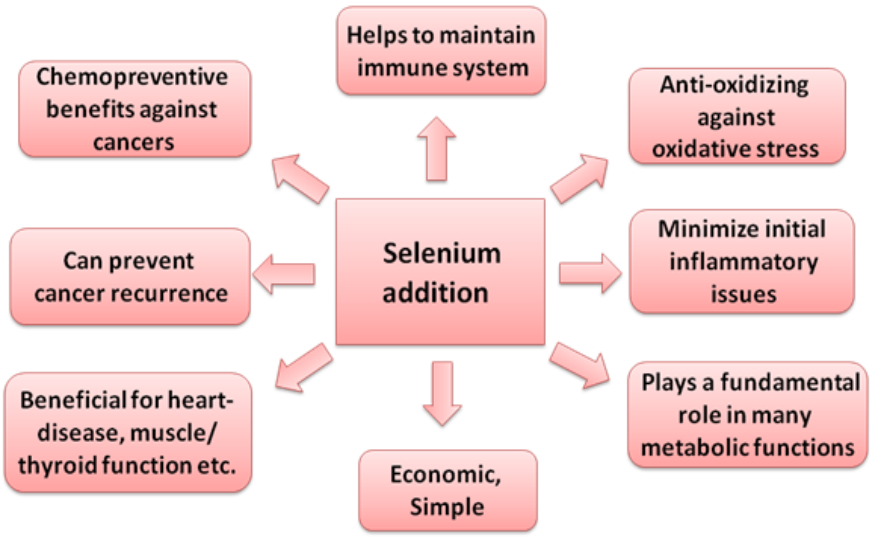
Figure S1: Summary of medical benefits of Se

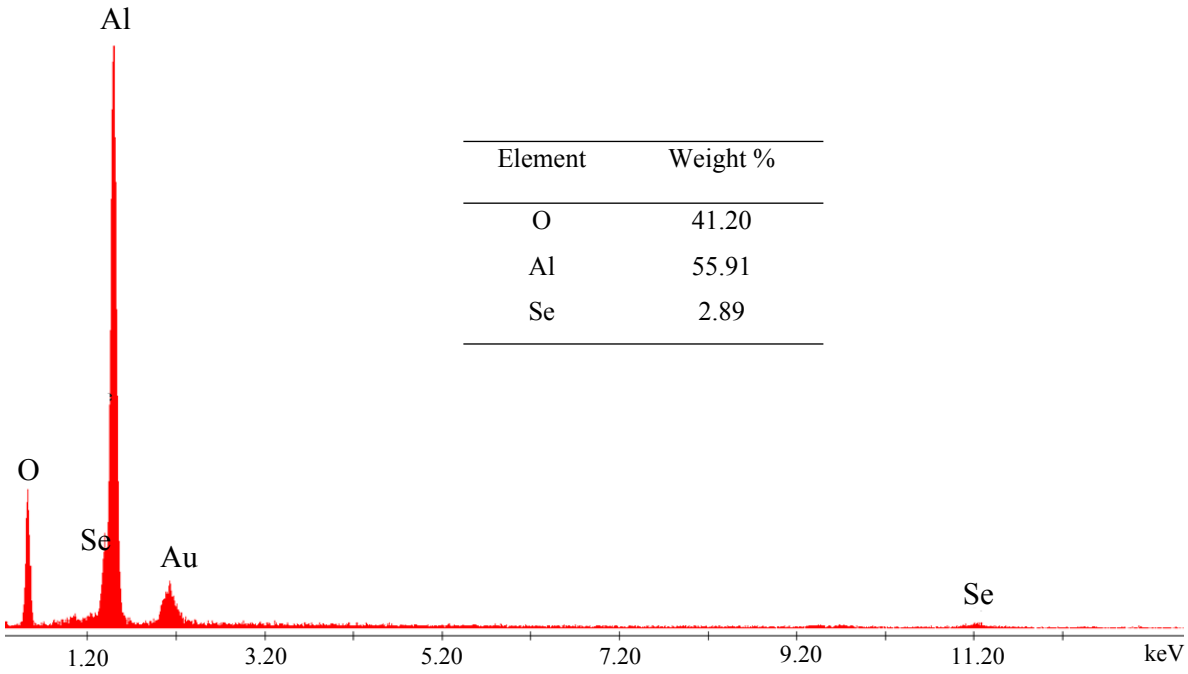


Cancer cell line research indicates a number of significant Se effects. A substantial body of animal-based experimental data indicates that Se protects against cancer. Its deficiencies are harmful to human health and that may increase the risk of cancer. Se can have unexpected advantages due to its anti-oxidizing and medicinal properties when used in acceptable levels. One major advantage is that it can potentially prevent cancer recurrence. Replacement of cancerous bone with implants is an important orthopedic procedure.[4]

Figure S2: Digital photographs of representative (A) ED and (B) CVD samples.

The images correspond to AAO after performing typical electrodeposition and chemical vapour deposition. Both processes resulted red colour formation on AAO. ED sample was gold coated prior to deposition. CVD Se deposited on AAO only (not on Al) and that can be due to the less adherence of evaporated Se on Al.

Figure S3: Representative selected area EDS spectrum of Se loaded AAO. A comparison of % Se detected with different Se loaded AAO is also provided.

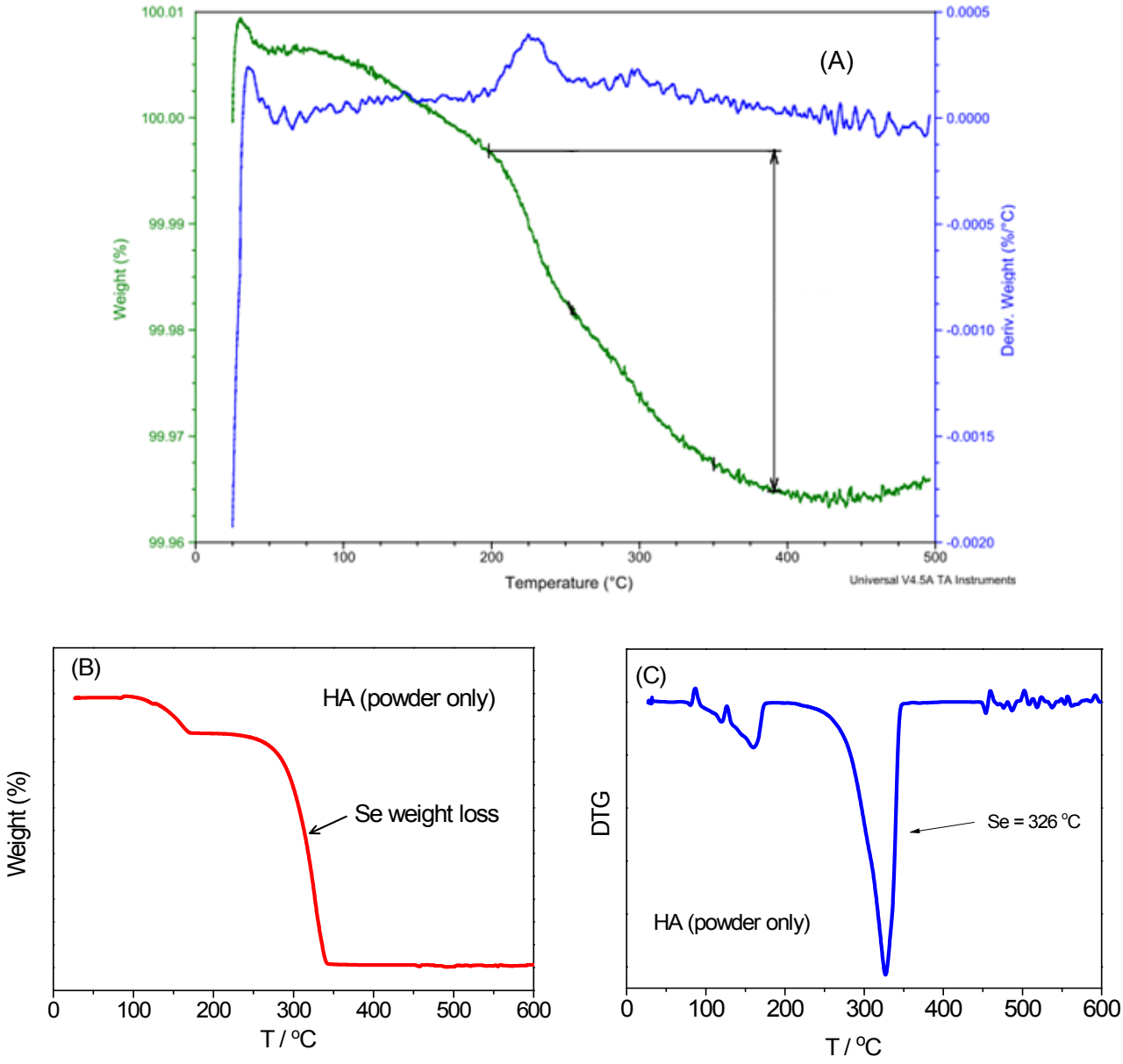


Element	Weight %
O	41.20
Al	55.91
Se	2.89

Type	Se (wt. %)
HA + Chitosan	1.74
DPDA + Chitosan	1.89
Se metal + Chitosan	1.79
ED Se (area - 1)	2.89
ED Se (area - 2)	1.69
CVD Se (area - 1)	3.19
CVD Se (area - 2)	1.61

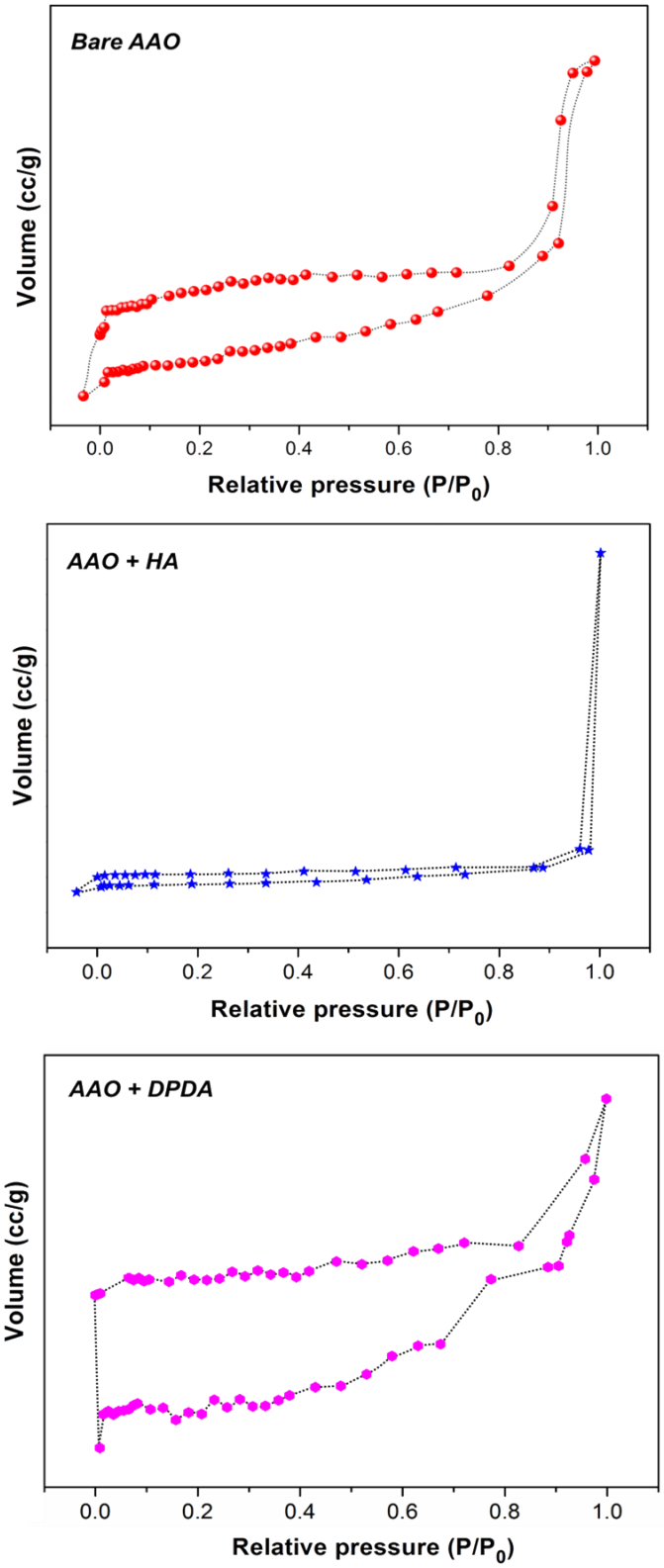
For chitosan composites, there was more or less homogenous Se loading where as both ED and CVD Se showed a few Se rich areas. Hence, % Se corresponding to two representative areas is provided for ED and CVD Se samples.

Figure S4: TGA analysis of **(A)** AAO loaded with Se; **(B)** & **(C)** Se powder.



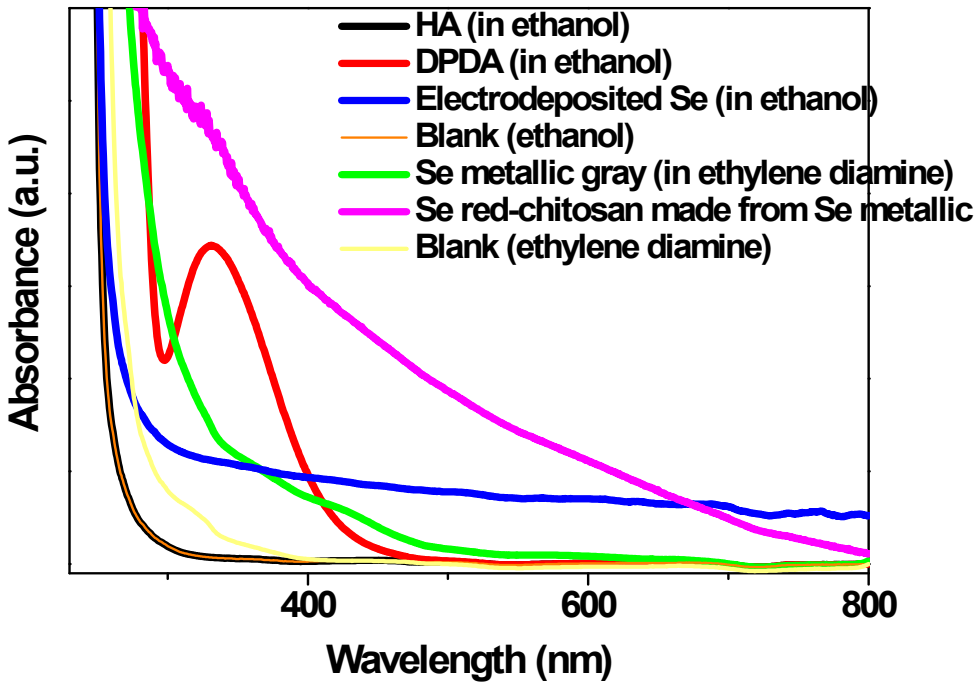
Representative TGA plot showing weight loss of Se loaded AAO is provided in (A). Weight loss between 200 and 400 °C was used for determination of the amount of loaded Se. Se typically shows weight loss at 320-330 °C (Figure S4 B & C, where TGA plots recorded for HA powder (without AAO) are provided). Similar TGA plots were obtained for other forms of Se, but with marginal variation of decomposition temperature. Being the loaded amount very small, a comparative weight loss between 200 and 400 °C was used to find the extent of Se loading.

Figure S5: BET curves of AAO before and after Se loading.



BET analysis of AAO before and after Se loading was carried out in order to determine the changes in the surface area as a function of loading. Here, representative studies were carried out using HA and DPDA loaded AAO. The measurements (adsorption and desorption curves) were carried out using Belsorp N_2 BET surface area analyser. BET analysis of AAO before and after Se loading shows a significant reduction in the surface area (i.e. bare AAO: 1.402 m^2g^{-1}, AAO + HA: 0.119 m^2g^{-1}, AAO + DPDA: 0.035 m^2g^{-1}). The pore volume measured was 0.003527, 0.000594, and 0.000523 cm^3g^{-1} for bare AAO, AAO + HA, and AAO + DPDA, respectively. Similarly, the mean pore diameter was reduced from 69.58 nm for bare AAO to 40.74 and 38.48 nm for AAO + HA and AAO + DPDA, respectively. Note that BET analysis for chitosan based samples was not carried out since it tends to block the AAO pores (see Figure 3).

Figure S6: UV-Visible spectra of Se solutions.



HA and DPDA were dissolved in ethanol. For ED Se, the precipitated Se in the electrolyte during an extended electrodeposition was filtered and the precipitate dispersed (by ultrasonication) in ethanol. Here, electrodeposition was performed under similar conditions as that employed for Se loading in AAO; but the deposition continued for longer time so that Se

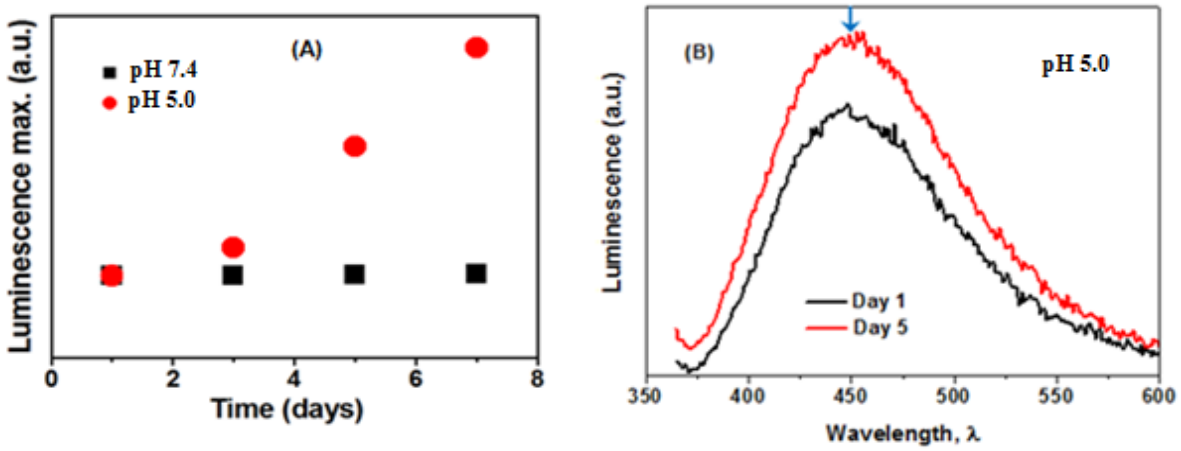
deposited in excess and the electrolyte turned red. The corresponding spectrum of the blank reference (ethanol) is also provided to compare its absorption with HA. For Se metallic pellet dissolved in ethylenediamine, a corresponding blank (ethylenediamine) was used. Se red-chitosan solution was prepared by adding dissolved Se metallic gray pellets (dissolved in ethylenediamine and diluted with ethanol) to chitosan-acetic acid solution and the spectra measured with reference to an equivalent blank.

Colorless solution of HA in ethanol (or HA-chitosan solution) does not show a characteristic absorption maximum corresponding to Se. Absorption at $\lambda < 250$ nm corresponds to the solvent, ethanol. Organic Se (DPDA, both in ethanol and chitosan) showed a characteristic absorption maximum at $\lambda = 360$ nm. ED red Se showed long wavelength range of absorption extending from 300 to 700 nm. The broad, featureless absorption obtained in this case can be due to the amorphous, aggregated Se clusters in the dispersed Se solution.[32] Metallic Se gray dissolved in ethylene diamine (not used for loading) also showed an extended absorption from 300 to 600 nm with an evident peak at $\lambda \sim 420$ nm. Red Se-chitosan solution obtained by adding metallic Se to chitosan-acetic acid also showed long wavelength range of absorption with a smaller peak at $\lambda \sim 300$ nm.

The spectral properties of Se can be different depending on the formation conditions. The typical structural arrangements of atoms in various allotropes of elemental Se are rings or chains of Se atoms, which can have different molecular compositions or chain lengths, ranging from Se_3 to Se_{12}.[33] It has been shown that the UV-Vis spectra obtained for three biologically derived Se samples varied considerably from species to species, and the Se nanosphere samples from *S. shriftii* exhibited a broadest absorption spectrum at $\lambda > 600$ nm.[34] Increased Se ring-ring interactions can cause a red shift in the absorption spectrum.[32]

The absorption maximum generally shifts towards red, and the peak intensity decreases with increase of the size of nanoparticles.[32] Se nanoparticles are known to exhibit a regular absorption maximum, in the wavelength region above 300 nm, only when the particle size is 100 nm, or more. Zare *et al.* reported UV-Vis absorption maximum at $\lambda = 240$ nm for biologically synthesized nanoparticles with Se particle size less than 100 nm.[35] UV-Visible spectra of Se nanospheres recovered from a culture broth gave a characteristic peak at $\lambda = 590$ nm and that corresponds to particle size of 182.8 ± 33.2 nm.[36,37]

Figure S7: (A) Variation of photoluminescence peak maximum of CVD Se deposited AAO. **(B)** Representative photoluminescence spectrum at two different time periods.

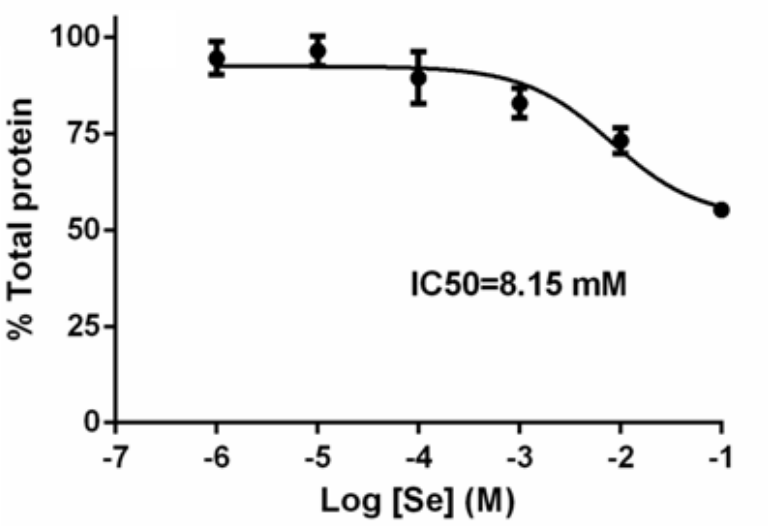


The variation of photoluminescence maximum (value corresponding to blue arrow mark in Figure S7 B) was used for comparison. The photoluminescence peak maximum remained almost the same in neutral pH whereas in acidic pH, the peak maximum showed a gradual increase with time (Figures S7 A). This can be due to the slow removal of Se from AAO in acidic pH and that in effect can increase the photoluminescence response of AAO. The photoluminescence maximum of CVD Se deposited AAO was lower than that of bare AAO (not shown) and that can be associated with the appreciable photoconductivity of Se,[17] and its attachment on AAO which results in masking of photoluminescence centers in AAO.[39] Before photoluminescence measurement, AAO washed repeatedly in distilled water so that a surface similar to pre-immersion was retained. A fluorescence spectrophotometer with Xe lamp as the excitation light source, at room temperature, and at an excitation wavelength of 350 nm was used.

Figure S8: IC50 value determined for HA.

The HOS osteosarcoma cell line was used to investigate biocompatibility and cytotoxicity of Se containing AAO samples. The cells were cultured in a modified minimal essential medium (α MEM, Gibco) supplemented with 10 vol. % fetal calf serum (Gibco), 10 mM HEPES (Gibco) and 1 vol. % penicillin (Gibco) at 37 °C in 5 % CO_2. The Se loaded AAO samples (in triplicate) were cut into a circle with diameter of 1.5 mm, gas sterilized and placed

into 24 well tissue culture plates (Nunc). HOS cells were seeded onto the samples and tissue culture plastic controls at a density of 5 x 10^4 cells/cm^2 and cultured for 24 h at 37 °C. After 24 h, the media was removed and protein was harvested from each sample or well by scraping in 30 µL lysis buffer (5mM EDTA, 5mM Tris-HCl and 0.5 % Igepal). The total protein was determined using the bicinchoninic acid assay (Pierce™ BCA protein assay) according to the manufacturer's instructions and expressed as a percentage of the tissue culture plastic control.



To determine the IC50 value, a range of HA concentrations (1 µM to 100 mM) were prepared in culture media. HOS cells were plated into 24 well plates at a density of 5 x 10^4 cells/cm^2 and cultured overnight. The media was then replaced with the Se-containing media (4 replicates per concentration) and the cells were cultured for 6 h, when cell death was observed in the cultures. The media was then removed and the protein harvested by scraping the cell monolayer in 30 µL lysis buffer. The total protein concentration was determined using the BCA assay and expressed as a percentage of untreated cells (without Se).