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Original article

# Study of accumulation behaviour of tungsten based composite using electron probe micro analyser for the application in bone tissue engineering

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## ABSTRACT

In this research, a proto-type study we have conducted, where we have synthesized tungsten based composite materials which are tungsten along with combined oxides of other elements like calcium, scandium, barium, and aluminium in the form of powder with bones powder of mice devised by high energy ball mill and later on fabricating high dense pellets by sintering by spark plasma. The particle sizes of the composite materials are found to be 1–2  $\mu\text{m}$ , as evidenced by the electron microscope, suggesting synthesized materials are of micron size. The quantitative and qualitative analysis of sintered pellets are well confirmed by electron probe micro analyzer (EPMA) and energy dispersive X-ray spectrometer (EDS) which illustrate the greater percentage of tungsten presents in the profound scan areas with other elements of the composite. The absence of pores across the 3D geometry suggesting dense sample, which is quite revealed by the X-ray tomography inspection. The prepared sintered pellets from the tungsten based composites are found to be  $\approx 99.5\%$  density with the observation of tungsten to be accumulated uniformly across the scan regions along with focussed hot spots as implied by EPMA. This study paves the way, to examine how the tungsten accumulation and the distribution with the other elements for future understanding in bone tissue engineering application and the in vivo specification of tungsten.

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## 1. Introduction

In recent times, with respect to various researches, it has been found the accumulation of tungsten in bones of mice with the aid of drinking water when exposed to the element (Hur and Reeder, 2016). These detections, by the team of biologists have paid the attention for the role of tungsten, how far considerable for the health risk factor for the human race (Kelly et al., 2013). Taken into consideration of materials, the transition metal tungsten is regarded having highest melting point, very good hardness,

and incredible density, which make it suitable candidate bioinorganic material to be used in medical, industrial, space and military application etc. (Oryan et al., 2014). When it is appraised about medical devices, medication equipments, and cutting tools etc, tungsten has found its place in our daily lives. However, with the view of many unique properties and advantages, there are some limitations have also been imposed with regard to vulnerability of air sourced tungsten dust to safeguard mine workers and the persons involved in processing of metals (Song et al., 2019). There are very few agencies, which also set limits on water-soluble compounds where tungsten was the essential element to address the broader section of the community at the risk of exposure. In the early 2000, there was a matter came into feature in Fallon, Nevada, when the scientists examined childhood leukemia cases, which was because of optimum levels of tungsten in the ground water supply in the city (Offner et al., 2019). This medical case paved the way to investigate the toxic effect and the carcinogen study of the tungsten and the accumulation in the bone of the material

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for the health consideration. So it has been a study of analysis in order to determine whether tungsten is a contaminant material or not, if it is consumed in the body (Nandi et al., 2010). Some recent studies highlighted the accumulation study of tungsten in the bones of mice by drinking water (Pei et al., 2019). There are some other materials like strontium and lead can also accumulate in the bones, but it was claimed to be separated using conventional chelation therapy (Arcos and Vallet-Regí, 2020; Ramya et al., 2012). However, this kind of therapy was found to be futile as some contents of the material was retained in the bone tissue even if the source is disconnected. This advocates substitute methodology or accumulation behaviour for tungsten in comparison to other heavy metals. It is very important to realize the biological insinuation of the accumulation of metals and to evolve a potential treatment approach, in order to determine if there is any selective deposition in certain regions of bone tissue, as well as to manifest the chemical form in that matter (Mitrán et al., 2018). Tungsten accumulation may have a diversity of inimical consequences, concern to the category of bone tissue in which it is available. In this regard, as per characterization, electron probe micro analyzer (EPMA) has been considered to be a non-destructive technique that has been appertained in different domains including physics, material science, forensics, geology and bio-technology to concurrently recognize and contiguously resolve various elements ranging from the micron to sub-micron scale (Pradhan et al., 2016). This methodology has been prosperously implemented to probe the accumulation of various heavy metals in bone, also it has been applied to govern the dimensional spread of bone-accumulated tungsten. The localized distribution of tungsten for the purpose of bone tissue engineering has been analysed for the case study of mice by exposing certain ppm of tungsten with the aid of drinking water for certain period of time, determined using another technique called synchrotron radiation micro X-ray fluorescence spectroscopy (Hur and Reeder, 2016; Mehrotra et al., 2019). The spectra were acquired to analyze the elemental distribution in each of the scanned regions. With this methodology, there was an observation of localized and centralized domains of tungsten in all of the main components of bone tissue. These analyses were prominent along the different length of bones across all exposure times. It was also found that the existence of tungsten in the marrow and cancellous tissue at the end of long bone (Bolt et al., 2016; Eivazzadeh-Keihan et al., 2019). Generally, the tissues are the site of requisite bone activities (Blair, 1998), such as development and immune cell establishment. Whether tungsten inhibits in these proceedings is under the examination, however, recent studies have revealed that mice exposed to tungsten have enhanced DNA deterioration in both bone marrow and white blood cell (Han et al., 2019; Venkatesan and Kim, 2010). Tungsten is also contained into the bone tissue which may influence the bone formation and coalition. It has been found that the excessive concentrations within the cortical tissue in relation to the bone marrow specify a mechanism of embodiment working against the concentration gradient of tungsten in blood (Xie et al., 2019; Souza et al., 2017). The analogous behaviour with respect to diversity of tungsten deposition, particularly noticeable in cortical bone tissue, has major indications for the mechanism to take place. This analysis is especially apparent when comparing the distributions of calcium and tungsten after the exposure of tungsten for few weeks. As per study it has been found using synchrotron radiation micro X-ray fluorescence spectroscopy (SR- $\mu$ XRF), calcium exhibits a relatively uniform distribution along with most of other trace elements along with tungsten. It has been also analysed that the localized tungsten deposition comprised during the bone development. This investigation has been conducted for different case studies for the mice bones. The analogous diversity is also found in case of zinc, which provides exceptional bone growth and as it is known to be deposit between

zones of bone (Hooshmand et al., 2014; Ibrahim et al., 2016). The diversity of gathering of both tungsten and zinc at the inner and outer edges of the bone tissue is likely to be linked to the variation of width, and thus enhanced remodelling activity, of the long bone in the case of young, growing mice. In our research, we have tried an attempt to prepare a proto-type of sintered pellets via powder metallurgy route, where we have synthesized tungsten based composite materials which are tungsten along with combined oxides of other elements like scandium, barium, calcium, and aluminium in the form of powder with bones powder of mice devised by high energy ball mill and later on fabricating high dense pellets by sintering by spark plasma. The proto-type of sintered pellets have been investigated via electron probe micro analyzer (EPMA), in order to examine the distribution of elements and the accumulation of tungsten for the given scan region. This proto type analysis will help for further study for the bone tissue engineering in order to carry out future study on accumulation behaviour of tungsten for the specific scan region in the bone. Recent studies have been carried out using synchrotron radiation micro X-ray fluorescence (SR- $\mu$ XRF) spectroscopy, but very few studies using electron probe micro analyzer (EPMA). Here we use EPMA for a proto-type study in order to examine how the tungsten accumulation with the other elements and the distribution for future understanding in bone tissue engineering application and the in vivo specification of tungsten.

## 2. Materials and methods

In this experiment, nano  $\text{Sc}_2\text{O}_3$  (scandium oxide) was prepared from the calculated amount of the glycine and scandium nitrate. Necessary care had been taken for the materials which were of analytical grade and high pure. For the purpose of better result, calculated amount of crystalline, white solid scandium nitrate ( $\text{Sc}(\text{NO}_3)_3$ , 99.7% pure) and crystalline, white solid glycine ( $\text{NH}_2\text{CH}_2\text{COOH}$ , 99.6% pure) of different batches were considered and then dissolved in a required volume of water to acquire transparent aqueous solutions. To remove the excess solvent, the deriving solutions were prepared in a glass beaker, subjected to thermal treatment on a hot plate at around  $120^\circ\text{C}$  with continuous uniform mixing, with the help of magnetic stirrer. It resulted in the viscous liquids in the form of gel from these homogeneously mixed solutions because of the process of thermal dehydration. For synthesizing adequate amount of powders, the viscous gels were subjected to heat treatment and evaporated constantly at about  $200\text{--}250^\circ\text{C}$ , for 20 min to 1 h. The  $\text{Sc}_2\text{O}_3$  powders were subjected for the calcination at  $1000^\circ\text{C}$  for 1.5 h in a retort furnace. In order to form the essential ingredients of scandate powder, nano sized  $\text{BaCaAl}_2\text{O}_3$  powder had also been synthesized. In our synthesis methodology, the individual materials of barium carbonate, calcium carbonate, and aluminium oxide were milled together in the high energy ball mill in powder form at 400 rpm in the occupancy of toluene with 20 mm diameter tungsten balls, so that the balls to powder proportion was maintained 10:1. After the said milling process for one day,  $\text{BaCaAl}_2\text{O}_3$  powder was subjected for calcinations at  $1200^\circ\text{C}$  for 12 h in the retort furnace and again the milling process was carried out for 5 h. The tungsten based composite materials were synthesized in the constitution of tungsten (80% by weight.), scandium oxide (10% by weight) and barium calcium aluminate (10% by weight) with bones powder of mice, pulverized all together for 8 h in high energy ball mill at 400 rpm, with 20 mm diameter tungsten balls in the occupancy of toluene. In our methodology, for the purpose of fabrication, the technique of sintering by spark plasma (Make: SPS-625, Fuji Electronic Industrial Co. Ltd., Japan) was deployed with the application of sintering pressure at 80 kN subjected to vacuum with  $\sim 3 \times 10^{-3}$  Pa and at sintering temperature

of 1450 °C with a holding duration of 5 min. The rate of heating was sustained constantly at 40 °C/minute and was experimented in optimum dense graphite die with diameter of size ~12.5 mm. This technique offers expeditious consolidation with utilization of pulse DC current to disseminate energy of spark plasma (Pradhan et al., 2016) among the constituents of the particles in the powder for making high dense samples with greater precision and invariability with shorter sintering time. Hence, sintering by spark has greater advantage in terms of tailoring of microstructure and to control grain growth. By this technology, very high dense sintered pellets had been prepared along with improved mechanical strength (density  $\approx$  99.5% measured by Archimedes principle), microstructures, and other special properties etc.

### 3. Results and discussion

The tungsten based composite powders of 8 h milled samples are examined through field-emission scanning electron microscopy (Zeiss, Carl Zeiss SMT AG) which is depicted in Fig. 1. The characterization has been conducted for different scan regions in order to understand the morphology and distribution of the constituents of the composite. In Fig. 1(a), we have analyzed the full scan region and in Fig. 1(b), we have chosen a small scan region. In this investigation, we find non-uniform distribution and spherical shape of the particles of nearly 1 to 2  $\mu\text{m}$  in size of the 8 h milled composite sample. It is quite evident from the EDS analysis of Fig. 1 that, the ingredients like calcium (essential of bone constituent), tungsten, barium, scandium, aluminium, carbon and oxygen are available and no other elements are detected in 8 h milled composite powder. The physical properties of the sintered specimens are also examined via density measurement. The bulk density of the sintered specimens is measured using Archimedes principle, taken into the consideration of weight of the specimens in the air and weight specimens in the water and then finally compared with the theoretical density. Applying simple rule mixture,

the theoretical density is calculated. At last, the density of the specimens is measured, from the ratio of bulk density and the theoretical density of the specimens. In our research, we have taken 10 sintered specimens for the reference for the density measurements and all the specimens show  $\approx$  99.5% density. The characterization of the fabricated tungsten based composite sintered samples is investigated through EPMA (JEOL Super Probe JXA8200). This examination is considered to be a very precise methodology for getting to know the availability of the ingredients quantity in the given scan region of the specimen. In this technique, the specimen ingredients can be comfortably recognized by analyzing wavelength dispersive spectra by gathering the X-ray released by the different elemental constituents, as the working principle of EPMA is based on the bombardment of the specimen by the high energy electron beam. Fig. 2 depicts the subjective analysis and quantitative availability of the tungsten based sintered specimen and its analogous electron microscopy image in the Fig. 2(a). It explicitly confirms the elemental ingredients and availability of scandium, tungsten, aluminium, calcium, barium, oxygen, and carbon in the given scan area as analyzed in the Fig. 2(b) and (c). From the analysis, it is quite clear that, the high percentage of tungsten presents in all most all the scan areas along with other ingredient elements of the tungsten based composite. Further we inspect by the confirmation with respect to the image, Fig. 2(b) that, from all the elements from the composite, scandium, barium and calcium mostly disperse across the grain boundary area of tungsten with the availability of other elemental species. It has been found that tungsten has been accumulated uniformly across the scan region and it has been focused in specific hot spots, as implied in EPMA analysis in Fig. 2(b). Also from the morphological analysis as per Fig. 2(a), it is quite evident that, as the temperature is very high around 1450 °C, there is a growth and agglomeration of the particles as a result, the pores seem to be non-available over the matrix, specifying dense samples. The sintered tungsten based specimen is further examined by X-ray tomography (XRadia/Zeiss VersaXRM

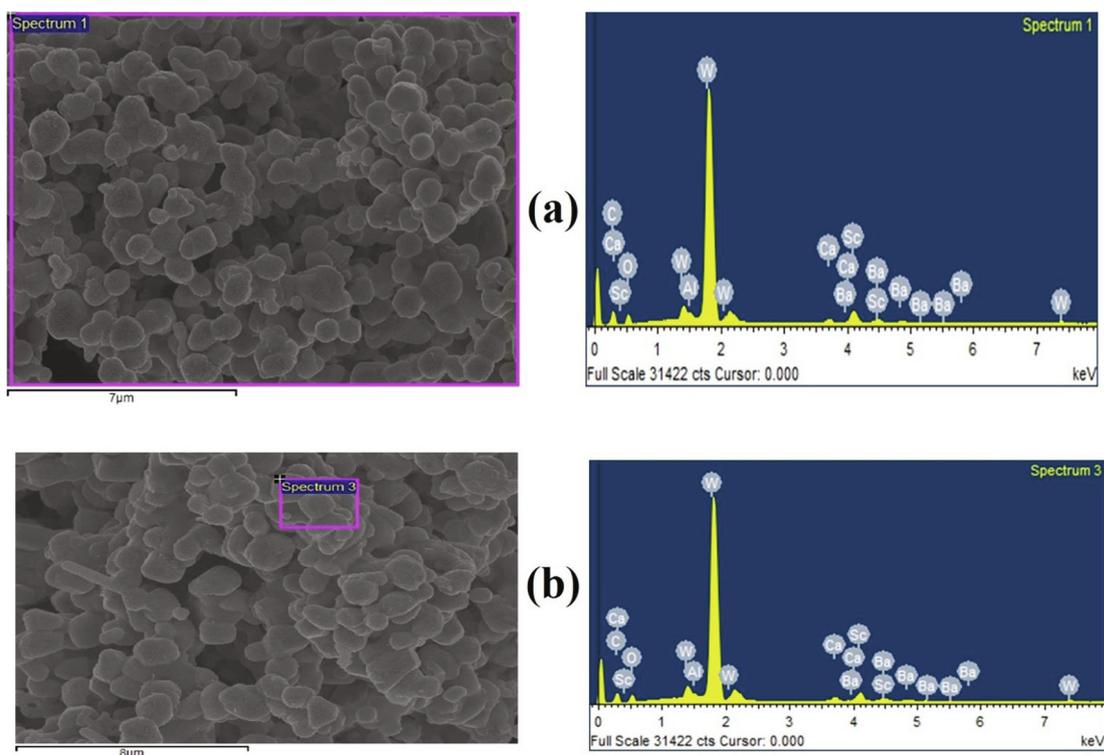


Fig. 1. Electron-microscopy images and analogous EDS analysis of (a) and (b) 8 h milled tungsten based composite powder sample.

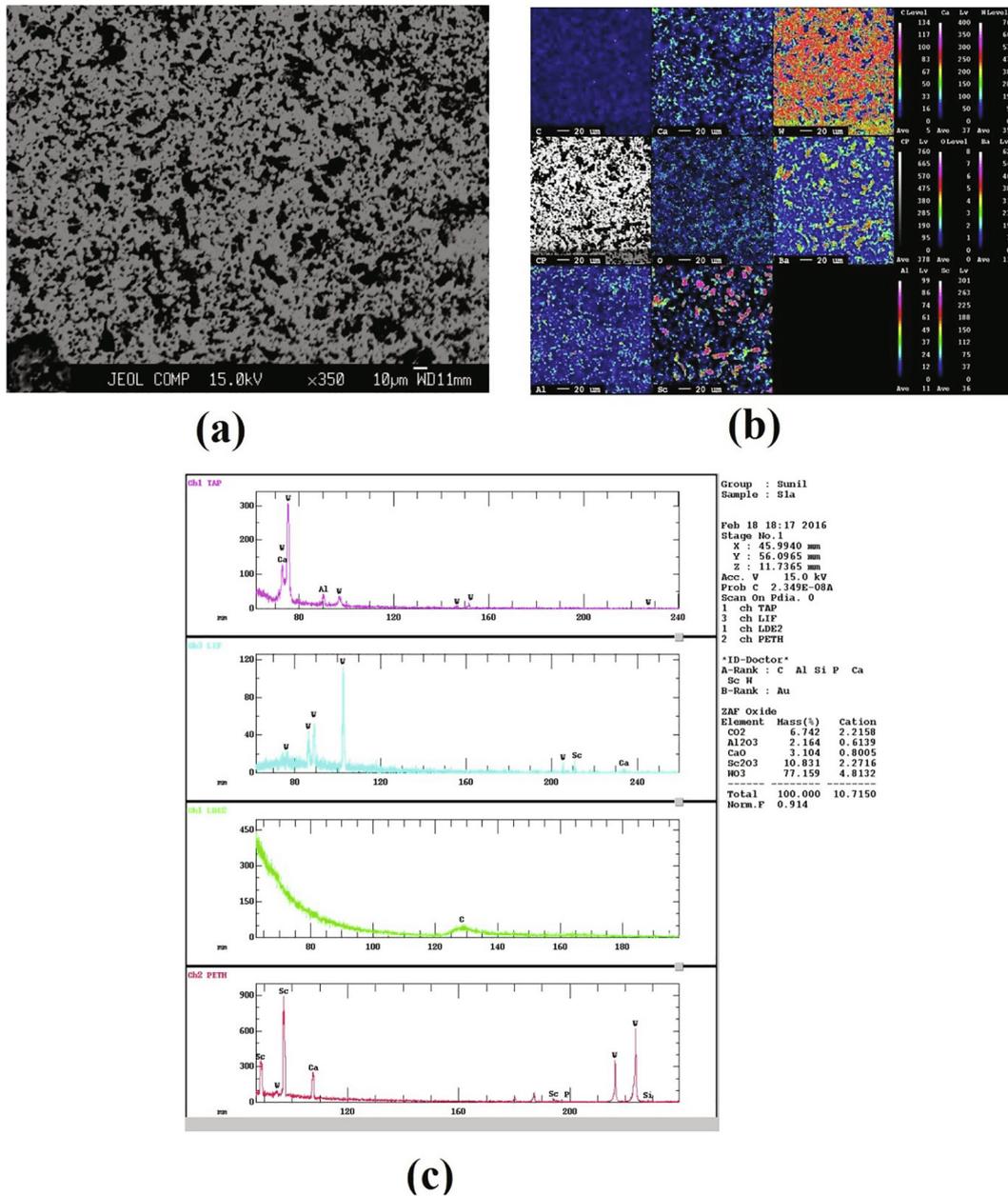
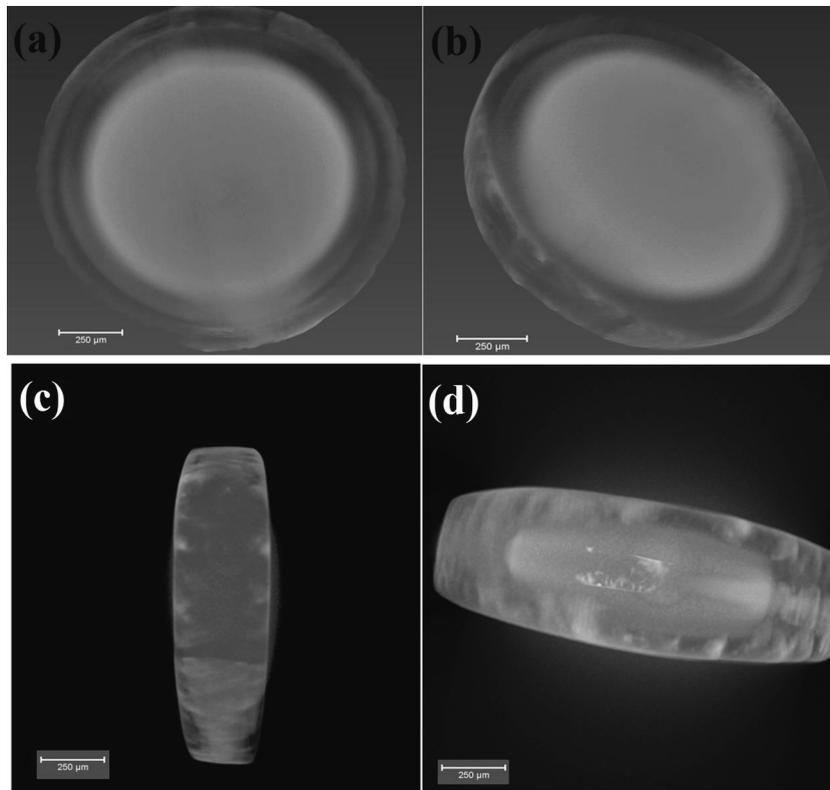


Fig. 2. EPMA characterization (b) and (c) of the sintered tungsten based composite specimen for the given scan region and the analogous electron microscopy image (a).

410) facility, which confirms the maximum percentage dispensation of tungsten element across the 3 dimensional regions as depicted in Fig. 3 in different tilt angles. In this instrument, there is a provision of charge coupled device (CCD) along with flat panel detector. Within the instrument, there is a specimen chamber, where the X- ray tube, sample stage and the detector are placed in aligned position. In our examination, we had maintained source-to-detector distance to be 290 mm. In various angular position, the projections of the sample are recorded by a two dimensional detector. The angular range is set to be of 360°, for the purpose of high power focusing mode along with background corrections for the reference images. The exposure duration is set to be 650 ms per projection. Images are taken into consideration on a 19504 × 1550 FP detector allowing 5 μm resolutions. The accelerating voltage and the beam current of X-ray source were held around 200 kV and 220 μA respectively with the diamond window

installed. The 3D analysis is eventually regenerated from 1500 projection slices using the InstaRecon software. Each voxel of the reconstructed image is cubic with 9 μm size. In our examination, we have taken into the consideration of process-property relationship in order to understand the distribution and morphology of the devised samples by powder metallurgy route. The fabricated tungsten based samples are studied with the high accelerating voltage as there is a substantial challenges with respect to strong scattering and absorption of X-ray from the material of higher atomic number (in our case tungsten). The micro-CT analysis clarifies the homogenous distribution of W matrix all over across the 3D geometry. It is also clear that the composite sintered samples are free from pores and also there is no realm of macro-exclusion. It is to be highlighted that, due to the limitation of the instrument, the distribution of tungsten matrix can only be segmented. It is clear from the given scan area that, the tungsten based sintered



**Fig. 3.** X-ray micro-computed tomography image of the sintered tungsten based composite (a) and in tilt angle image (b-d).

specimen is completely free from any kind pores and any type of micro segregation, identifying dense samples, which is quite correlated with the density measurement by Archimedes principle.

#### 4. Conclusion

Using powder metallurgical route, a proto-type study is conducted, where, the tungsten based composite powders with bone powders of mice are prepared and deploying sintering by spark plasma the pellets are fabricated. Different micro-structural studies like electron microscopy and EDS are quite satisfactory and confirm the constituents of the tungsten based composite materials. The densities of all the sintered samples are found to be  $\approx 99.5\%$ . The EPMA analysis further corresponds to the actual presence of the ingredients of the composite along with 3D analysis with respect to X-ray tomography. It has been found that tungsten has been accumulated invariably across the scan regions and also has been focussed in certain hot spots. This study paves the way, to examine how the tungsten accumulation and the distribution with the other elements for future understanding in bone tissue engineering application and the in vivo specification of tungsten.

#### Declaration of Competing Interest

All the authors hereby declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this research paper. So on behalf of all authors, the corresponding author states that there is no conflict of interest.

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