INTRODUCTION:
Radiation therapy for malignancies of neck and head has adverse side effects on adjacent non-cancerous bone tissue. Osteoradionecrosis of the mandible, which is irradiated in order to reach the target tissue, is a major complication. Osteoradionecrosis occurs because of radiation-induced damage to the osteogenic cells [1], resulting in blunting of the remodeling and resorption processes. Amifostine, is a radioprotectant whose mode of action resides in the scavenging of radiation-induced free radicals [2]. In the only reported study [3], it was shown that, Raman spectra provide signatures of changes in both mineral and matrix caused by osteoradionecrosis of the mandible. We hypothesized that Raman spectroscopy would allow us to evaluate the protective effects of Amifostine on bone chemical composition, and provide insight into the partial remodeling that occurs. We test that hypothesis on a rat model of irradiated mandibular distraction osteogenesis.

METHODS:
Fifteen Sprague Dawley rats were randomly assigned into 3 experimental groups: Control (n=5), irradiated (XRT, n=5), Amifostine treated (AMF, n=5). Both the XRT and AMF groups underwent human equivalent radiation dosage of 70 Gy. The AMF group received a prophylactic dose of Amifostine subcutaneously 45 minutes prior to radiation. All animals survived. After a 56-day post-radiation period, the animals were sacrificed. The left hemimandibles were harvested and frozen at -20 °C until Raman analysis. Our Institutional Committee for the Utilization and Care of Animals approved all studies.

Raman spectra (microprobe, 785 nm excitation) were collected from the region of interest on the hemimandible surface. Both bone mineral and matrix-specific Raman bands were analyzed and band intensity ratios [4], such as carbonate/phosphate (1070/958 cm\(^{-1}\)) and phosphate/matrix (958/(854+873) cm\(^{-1}\)), cross-link or matrix maturity (1660/1690 cm\(^{-1}\)) were calculated using GRAMS/AI software (Figure 2). Raman difference spectra of [XRT] minus [Control] and [AMF] minus [Control] were obtained to show spectroscopic differences between XRT, AMF and Control (Figure 1).

One-way ANOVA was utilized for assessing the difference in mineral and matrix composition of murine mandible in different groups. Statistical significance was considered at p≤0.05.

RESULTS:
Figure 1 summarizes major spectroscopic differences. In the XRT-treated rats, the intense phosphate P-O band at 958 cm\(^{-1}\) shifted to a higher wavenumber, whereas smaller intensity and wavenumber changes were observed in other mineral and matrix bands (Figure 1 (b)). The effects were similar to those previously reported for human subjects [3]. Band position shifts and intensity changes were also observed in matrix collagen bands. Most of the differences disappeared in the spectra of Amifostine-treated animals (Figure 1 (c)). For example, statistical analysis of the band ratio of carbonate/phosphate intensities and full width at half maximum (FWHM) of phosphate \(\nu_1\) band showed no significant difference between AMF and Control. These parameters for both groups were significantly different from XRT. The significant difference in phosphate/matrix ratios were observed for different groups. No difference in 1660/1690 cm\(^{-1}\) ratio was found for AMF and XRT while they were significantly different from Control.

DISCUSSION:
Raman spectra showed radiation-induced changes to mineral and matrix composition. Surprisingly, the tissue becomes overmineralized, but with an abnormally crystalline mineral, as evidenced both by the shift to higher wavenumber and a decrease in the width of the band at 958 cm\(^{-1}\). Within experimental error Amifostine returns these parameters to their Control values. There may still be subtle abnormalities in the mineral which are suggested by some remaining difference in the phosphate bending vibration. The cross-link or maturity parameter increases after irradiation, but Amifostine does not return this parameter to its Control value.

The collagen matrix that initially forms after mechanical injury to bone tissue is highly disordered and results in the weak woven bone. It is likely that a similar disordered tissue is formed in response to radiation damage, with respect to limited angiogenesis in the tissue. This conjecture is supported by the similarity of the collagen cross-link or maturity parameter (1660/1690 cm\(^{-1}\)) in the XRT and AMF groups. Further test of this conjecture is needed.

Figure 1. (a) Typical Raman spectrum of murine mandible with the main band assignments for bone mineral and matrix; (b) Raman difference spectrum of [XRT] minus [Control]; (c) Raman difference spectrum of [AMF] minus [Control].

Figure 2. Comparisons of carbonate/phosphate, FWHM of phosphate band at 958 cm\(^{-1}\), phosphate/matrix and 1660/1690 cm\(^{-1}\) for XRT, AMF and Control. Asterisk represents p≤0.05.

SIGNIFICANCE:
Raman spectroscopy elucidates the changes caused by radiotherapy and the strengths and limitations of free radical inhibitors as radioprotectants. Ongoing development of non-invasive Raman spectroscopy of bone tissue in several laboratories suggests that the technique may be used to monitor radioprotectant function in human subjects and monitor changes induced by radiation in any patient undergoing radiotherapy regardless of the pathology.

ACKNOWLEDGEMENTS:
Funding through NIH/NIAMS grant R01AR055222

REFERENCES: