INTRODUCTION: Osteosarcoma is the third most common bone cancer among children and adolescents. Osteosarcoma causes large tumors embedded in the bone and requires removal, leaving critical size defects. Not only does the removal of tumors embedded in the bone tissue result in critically sized defects, the risk of residual malignant cancer cells is also a serious concern. Thus, natural medicinal compounds incorporated with the three-dimensional (3D) printing of calcium phosphate scaffolds acting as a local drug delivery system present a promising candidate to facilitate bone healing and chemoprevention.[1] 3D printed calcium phosphate scaffolds with interconnected macro and micropores can promote and guide bone regeneration due to their appropriate porosity and mechanical properties, especially for patient specific defect repair applications. Epigallocatechin gallate (EGCG), the main component extracted from green tea, is one of the most promising candidates for this treatment because of its wide range of therapeutic efficacy for anti-inflammation, chemoprevention, and osteoporosis. EGCG induces apoptosis in osteosarcoma through upregulating microRNA-1 (miR-1) and inhibiting the nuclear factor kappa B (NF-kB). EGCG has also been shown to enhance angiogenesis by upregulating the vascular endothelial growth factor and osteoblast proliferation via the bone morphogenetic protein 2 (BMP-2) signaling pathway. In addition, it inhibits osteoclast activity through the NF-κB pathway. Hence, the 3D printed scaffold for localized EGCG delivery may provide notable improvements in anti-osteosarcoma properties, osteogenic ability, and vascularization formation (Fig.1).

METHODS: Scaffold preparation: TCP disc and 3D printed scaffolds are fabricated using β-TCP powder prepared by the solid-state synthesis method. TCP discs are prepared using a uniaxial press and the 3D printed scaffolds are fabricated by a binder jet printer.

In vitro EGCG release: Drug release of EGCG from the TCP discs is studied in an acetate buffer solution (pH 5) and phosphate buffered saline (pH 7.4) to mimic post-surgery acidic environment and the physiological environment of body, respectively. In vitro biological characterization: For in vitro cell culture studies, EGCG is loaded on the 3D printed β-TCP scaffolds. For osteosarcoma cell culture studies, MG-63 osteosarcoma cells (ATCC) are seeded onto the scaffolds at a density of 3x10⁴ cells/scaffold. The anti-cancer effects of EGCG in MG-63 cells are examined by cell viability assay (MTT) and cellular morphology. Matrigel coated plates are prepared and seeded with 7.5x10⁴ Human Umbilical Vein Endothelial Cells (HUVECs, ATCC) for in vitro tube formation assay. The formation of capillary tubes by endothelial cells is examined by an optical microscope at specific times.

Statistical analysis: All statistical analyses are performed using one-way ANOVA and post-hoc Tukey-Kramer analyses. All measurements are made in triplicate and P value ≤ 0.05 is considered as statistically significant.

RESULTS: Table.1 shows the EGCG release from TCP discs at pH 5.0 and pH 7.4. EGCG exhibits a low release and achieved a plateau range in pH 5.0, whereas it shows a higher initial burst release in pH 7.4. After 21 days, 74.8% release of EGCG is achieved in phosphate buffered saline, while only 33.25% of EGCG is released in pH 5.0 in the system. To evaluate in vitro anticancer activity, MG-63 osteosarcoma cell attachment, proliferation, and viability are tested. Osteosarcoma cell viability is significant difference between control and EGCG loaded scaffolds at days 3, 7 and 11 (Fig.2-a). The cell viability of the osteosarcoma cell is 45% and 66% lower with EGCG than control at days 7 and 11, respectively. EGCG stimulates tube formation at as early as 3 hours in HUVECs grown on Matrigel, compared to the control 3D printed TCP scaffolds, which shows few tubes and minimal network among the branches (Fig.2-b).

DISCUSSION: From the in vitro drug release study, EGCG shows an initial burst release for 1 day followed by a sustained release of the drug at pH 7.4. It clearly indicates that the pH of the release media has a strong influence on EGCG release kinetics from β-TCP discs. Considering the pKa values of EGCG, including 7.64-7.75 and 8.0 which are near the pH of the phosphate buffer solution, the phenolic hydroxy groups of EGCG are easily deprotonated by physiological pH (pH 7.4). EGCG has anti-carcinogenic effects in MG-63 cells, as shown in fig. 2-a. Reduced cell viability is detected in presence of EGCG at all time points. This result can be assessed by upregulation of miR-1 and suppression of the transcription factor NF-kB. Although studies report that EGCG inhibits tumor angiogenesis, it induces vascularization in normal tissues. This result is in agreement with previous study that EGCG enhances vascular endothelial growth factor expression. This feature indicates that EGCG has a bidirectional action on angiogenesis, which supports enhanced tube formation at early time points by an EGCG loaded 3D printed scaffold (Fig.2-b).

SIGNIFICANCE/CLINICAL RELEVANCE: Localized delivery of EGCG from 3D printed scaffolds has deleterious effects on osteosarcoma proliferation and enhanced angiogenesis in early stages suggesting a new approach in post-surgical patient specific defect repair applications.