Tiny Materials with Huge Potential: Developing Hydrogel-Based Therapies for Osteoporotic Bone Repair

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INTRODUCTION: Osteoporosis, characterised by depleted bone mass and disrupted bone architecture due to impaired bone remodelling, is the most prevalent metabolic bone disease in the world, causing fractures worldwide at a rate of one every 3 seconds, exceeding health care costs of € 37 billion each year[1]. Osteoporotic vertebral fractures (OVFs) are the most common complication of osteoporosis and patients determined to have OVFs are 5 times more likely to suffer secondary vertebral fragility fractures[2]. The clinical gold standard of care for OVFs is vertebroplasty and kyphoplasty, whereby cement is injected into the damaged vertebrae. These cements are not biodegradable and often leading to complications such as cement leakage and appearance of secondary fractures in adjacent diseased vertebrae[1]. The main aim of this study was to develop an advanced mechanically robust biomaterial technology to repair & restore structural integrity and function of disease-damaged bone. There is growing evidence that strontium (Sr) influences bone cells and bone metabolism *in vitro* and *in vivo* [3]. Therefore, inspired by the mechanical properties of single wall carbon nanotubes (SWCNTs) and the biological advantages of Sr, this work aimed to develop an innovative biomaterial strategy whereby SWCNTs were integrated into Sr loaded nanohydroxyapatite and further incorporated into chitosan-collagen (CS-COL) matrix to formulate mechanically robust hydrogels with advanced osteogenic capabilities for osteoporotic bone treatment.

MATERIALS AND METHODS: SWCNTs were functionalized with Sr loaded (0, 2, 5, 10 wt%) nano hydroxyl apatite particles (Sr-nHA) using co-precipitation method. SWCNTs@Sr-nHA decorated thermoresponsive CS-COL injectable hydrogels were prepared, crosslinked using β-glycerophosphate followed by freeze-drying[4]. Physicochemical properties was assessed by using XRD, FTIR, SEM, TGA, DSC,TEM techniques, Degradation, Calcium and Sr release profile was checked in PBS/media and mechanical properties was tested on a Zwick Roell testing machine using 5N load. Cytotoxicity and proliferation of rat mesenchymal stem cells with prepared hydrogels was assessed using lactate dehydrogenase activity, Alamar blue and PicoGreen assay. Osteogenesis was assessed via quantitative RT-PCR to detect key osteogenic markers, ALP activity and calcium deposition. RAW 267.4 cells were used to evaluate the osteoclastogenesis effect of Sr doping via TRAP activity and RT-PCR to detect key osteoclastogenic markers. Antimicrobial potential was evaluated with gram +ve microorganisms. Finally, our freeze-dried hydrogels were evaluated in an *in vivo* rat closed femoral defect model.

RESULTS: Mechanically robust SWCNTs were functionalized with Sr-nHA to investigate the effect of therapeutic ion on bone metabolism(Figure 1). The physiochemical characterization represent presence of calcium phosphate phases and Sr compound was not observed as separated phase, an indication of a complete substitution of Sr in the prepared hydrogels. Incorporation of Sr^{2+} ion resulted in the broadening and shifting of phosphate band in FTIR spectra due to the reduction in the degree of crystallinity. Hydrogels were found to mechanically robust, thermoresponsive (Figure 1) and non-toxic with enhanced osteogenic differentiation and promote mineralized matrix deposition with increasing content of Sr, and decreased TRAP activity for RAW 264.7 (Figure 2). This shows the repair & restore structural integrity for disease damaged bone without the need of added additional therapeutics. Effective release of Sr^{2+} ions from hydrogels can be exploited to treat bone defects provided that the *in-vivo* Sr^{2+} concentration is kept below the toxic limit. Our results have shown that the release of Sr^{2+} ion reaches 6ppb for 10wt% Sr after 3 days, which also enhanced antimicrobial properties for +ve organism S.Aureus (Figure 2). Following on this, freeze-dried hydrogels were implanted in rat femoral defect. Newly formed bone at the defective site was observed after 8 weeks of implantation and measured by using BV/TV values and H/E staining (Figure 3). The results of the present study provide evidence that prepared hydrogels are osteoconductive and osteoinductive in nature. Statistical analysis of data (N=3) was performed with one-way analysis of variance (ANOVA) and p-values less than 0.05 were considered significant.

DISCUSSION: Degree of crystallinity of nHA phase in the Sr-nHA samples decreases with increasing Sr^{2+} substitution, which can be attributed to increased lattice strain caused by larger Sr^{2+} ion replacing the smaller Ca^{2+} ion in the nHA lattice. SWCNTs maintain the high mechanical strength for hydrogels. In the *in-vitro* study, rMSCs attached, proliferate and differentiate on the freeze-dried hydrogels, which upregulates osteogenic gene expressions by ~ 5 fold and calcium deposition while downregulating RAW264.7 cells with addition of Sr. It implies that Sr positively regulate bone remodeling to promote osteoporotic bone healing. μCT analysis of newly formed at 8 weeks after implantation showed the increase in newly formed bone volume and H/E staining showed the integration of bone within the freeze-dried hydrogel and defect. This work proves the reparative potential of the developed therapeutic Sr loaded hydrogel influences bone cells and bone metabolism *in vitro* and *in vivo.*

SIGNIFICANCE/CLINICAL RELEVANCE: Formulated hydrogels show significant potential for bone repair, to tackle a devastating clinical orthopaedic challenge for which there is currently no reparative treatment. These hydrogels has the potential for effective reparative treatment for OVFs, in the form of novel mechanically robust hydrogels with enhanced anabolic action while promoting osteogenesis and inhibiting osteoclastogenesis both *in vitro* and *in vivo*.

REFERENCES: [1] Svedbom et al., 2013 [2] Sözen et al., 2017 [3] Marie et al., 2001,[4] Kaur et al.,2021

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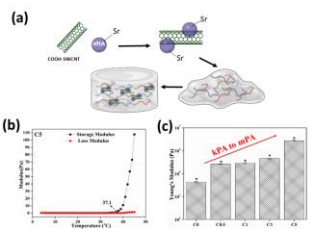

Figure 1: (a) Incorporation of functionalized SWCNTs into (b) thermoresponsive hydrogels (c) increased Young's Modulus of hydrogels by 63%, closer to that of trabecular bone

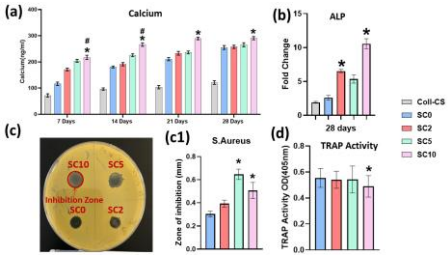

Figure 2: Increasing concentrations of Sr-nHA significantly increased (a) calcium deposition, (b) ALP gene expression, (c) antimicrobial potential against S.Aureus (c1) zone of inhibition and (d) decreased osteoclastogenic TRAP activity of rMSCs

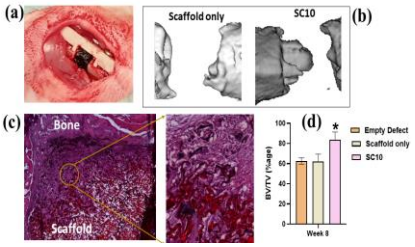

Figure 3: In-vivo femoral defect model (a) implanted freeze-dried hydrogels (b) μCT image (c) H/E staining and (d) BV/TV values after 8 weeks of implantation.