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An opinion on hydroxyapatite based bio-composites as bone-scaffolds

KANTESH BALANI*
Professor, Department of Materials Science and Engineering, Indian Institute of Technology Kanpur, Kanpur-208016, Uttar Pradesh, India

Abstract: Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is regarded as one among most bioactive materials for bone and hard-tissue replacement due to its chemical and structural similarity as that of apatite with Ca/P ratio of 1.67. But, the use of HA is limited due to its poor fracture toughness to the order of 0.5-1.5 MPa.m$^{1/2}$. Therefore, usually, some additives, such as Al_2O_3, YSZ, ZnO, Fe_3O_4, TiO_2, Ti, Ag, carbon nanotubes (CNTs), Ti, etc have been incorporated. It is observed that the metallic reinforcement is a better toughening agent than the ceramic reinforcement, but the release of metal ions may also hamper the key metabolic pathways of human cell. Further, β-tricalcium phosphate (β-TCP) and bioglass addition can be used for attaining controlled resorption of material under in vivo conditions so the natural bone can replace the artificial scaffold during healing process. Many additives, such as Ag, ZnO, CuO, TiO_2, etc have been incorporated to provide antibacterial efficacy to the scaffolds. The aspect of antioxidant activity obtained from aliovalent ceramics (such as CeO_2) may also assist in expedited healing. The design of porosity at multi-length scales can also be envisaged as means of incorporating cell-material interaction at bulk scale (~150-250 μm size, for vascularisation), at micrometer length scale (~10s of μm for cellular alignment) and at molecular length scale (~ few nm for surface protein interaction with implant substrate). Hence, the onus is on interdisciplinary biomedical engineers to aspire and design multifunctional bone-scaffolds with required mechanical integrity, antibacterial efficacy, bioactive response, biosorption for accommodating natural healing, and inducting expedited restoration.

Keywords: *Hydroxyapatite; Bone-scaffold; Toughness; Porosity; Protein Adhesion.*

INTRODUCTION

Healthcare advancements manage the ageing population and assists in retaining the social activity via offering total-hip joint arthroplasty (THA). For year 2015, with more than 12 lakhs THA annual surgeries [1], 4.7 lakh THA surgeries are performed in India [1]. And surgeries are on rise, and THA in the United States is ~ 1.2 lakhs in year 2015 [2], and, it is expected that around 10 lakhs may need such replacements in the year 2020. The success of hip implant is mainly governed by completing 15-20 years of successful service without any failures and with proper osseointegration (i.e. bone growth in the adjoining area of the femoral stem of hip implant). As the bioactivity is expressed in terms of osteogenesis (bone-formation), osteoconduction (bone-growth), to result bone healing (or osteoinduction) and osseointegration (integrating artificial implant with natural bone). Thus, from material selection to result bone formation requires a bioactive material like hyhydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$)/ β-Tricalcium phosphate (TCP) or bioglass (BG). Bioglass is typically calcium-sodium-phosphosilicates, and their use is limited by their very low fracture toughness and rapid dissolution in aqueous medium (and loss of structural integrity before natural bone formation). It is important to mention that the crystallinity and material resorption are very important aspects to decide on the applications. For example, high crystallinity of HA may prompt its application in load-bearing application and where the bone-remodelling may be minimal in elderly persons, whereas BG and β-TCP may be very attractive for porous-scaffolds and where material-resorption will be replaced by new bone cells, especially in younger patients. Nonetheless, these concepts have evolved for engineer to appropriately choose the composition and design the composites into complex shapes as porous/ 3-D printed scaffolds. Towards achieving osteoconduction, the introduction of macro-porosity assists in letting formation of fibrous tissues and accommodating vascularisation. The osseointegration highly depends on the surface of the implanted surface, and the assimilated surface bioactivity and continuous porosity allows formation of a unified interface between an implant and natural bone. The biodegrading species like bioglass, amorphous

* Corresponding Author Email : kbalani@iitk.ac.in

calcium phosphate or Mg-based alloys may also be utilised for replacing the implant material with natural bone in due course of time. It may also be mentioned that the healing process depends a lot on the metabolism and age of individual as the healing can be very rapid in young patients (and use of biodegradable materials becomes feasible), whereas the delayed or restricted healing process in elderly persons may mandate the use of bio-inert materials and the implant material may have to be completely bio-inert in making up for the lost functionality.

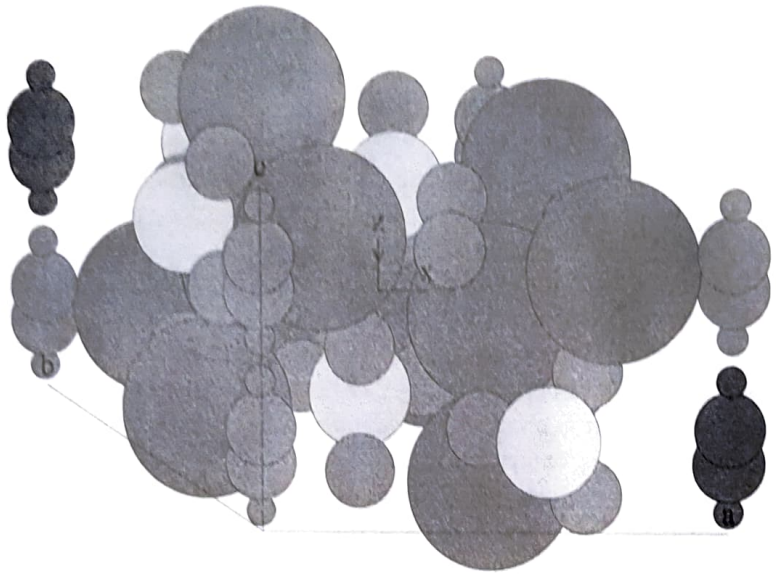

Fig. 1: Schematic of hydroxyapatite crystal showing Ca atoms (pink), oxygen atoms (green), phosphorous (yellow) and hydrogen (orange).

For the same, hydroxyapatite Fig. 1 is deposited (usually via plasma spraying) for achieving surface bioactivity on the surface of femoral stem and assisting bone-integration after THA. Otherwise, a bone-cement (called polymethyl meth acrylate, PMMA) is utilized as glue to attach the femoral stem with the surrounding bone. But, due to lack of bone-material interaction, this bone cement loosens with time and mandates a revision surgery, which is highly painful and costlier. Currently, plasma sprayed hydroxyapatite[3] is the only industrially scalable coating on femoral stem that is approved by Food and Drug Administration (FDA). Thus, bioactive nature of HA allows enhanced osseointegration due to its chemical similarity with that of apatite found in bone and teeth (Ca/P ratio of 1.67)[4].

TOUGHENING EFFECTS

Brittleness of HA is one of the major concerns (fracture toughness to the tune of ~ 0.5-1 $MPa.m^{1/2}$,[5] , thus, many researchers have proposed multi-pronged approaches of utilizing various reinforcements (e.g. bioinert Al_2O_3[6],Yttria stabilized zirconia (YSZ)[7], or bioactive carbon nanotubes (CNTs)[8] in order to enhance its fracture toughness. Ceramics are known to be brittle, nonetheless a limited toughening enhancement in ceramics can be obtained by grain size refinement, incorporating micro-porosity (to blunt/terminate the propagating cracks), crack branching, incorporating pinning agents, adding phase-transformation toughening agents, and inducing crack-bridging phenomena. CNTs have elicited crack deflection, crack-branching, CNT-pull-out and crack-bridging mechanisms to sustain enhanced damage tolerance[8]. It may be noted that Ag and other metallic reinforcements have shown to be a superior additive in achieving enhanced toughening even when compared to that of CNT[9, 10]. Herein, with CNT reinforcement, the fracture toughness is ~ 1 $MPa.m^{1/2}$, which increased to above 2.5 $MPa.m^{1/2}$ upon addition of Ag[9]. As the surface requires bioactivity, whereas the bulk requires structural toughness, a mere layering of these two composites will not work. Thus, an attempt of achieving surface bioactivity with HA-dominated surface and YSZ-bulk was interfaced with Al_2O_3-YSZ based cushioning layer as a functionally graded structure in sufficing both the requirements[11].

ANTIMICROBIAL EFFECTS

In order to extend the functionality of the bone-scaffold, it is preferred to add antibacterial agents to avoid post-surgical infections. Though the post-surgical infections are usually to the tune of 5%, but the pain and trauma to the patient can be twice as that of first surgery and the cost of revision surgery can be as high as up to 2-3 times. Thus, antibacterial agents such as Ag, Cu, Co, TiO_2, ZnO, Fe_3O_4, etc have been incorporated. On one hand, like for Ag, its ions permeate through bacterial membrane and denature DNA, whereas agents like ZnO or CuO can assist the release of reactive oxygen species (ROS) and rupture the bacterial wall. These killing mechanism of bacteria also extend to the use of AMPs (anti-microbial peptides) inducting opsonisation (of formation of localised porous regions in bacteria) and hamper their key pathways and eventually kill the bacteria. Further, an enhanced functionality with incorporation of ferromagnetic (Fe_2O_3/Fe_3O_4)[12] or antibacterial ZnO [13,14, 15], Cu, Ag etc.[16] have been incorporated in HA matrix. Utilisation of processing in controlling the incorporation of Cu in HA matrix [17] or site specific anti-bacterial efficacy of Zn [18] or Co [19] in HA matrix or even the effects of co-doping of Zn and Co in HA matrix [20] well elucidate and establish Co as superior antibacterial agent (than Zn), and also enunciate a stronger release of Co when doped at Ca site compared to that at OH site of HA.

EFFECT OF POROSITY

Though there have been various reports on controlling porosity on isolated micro-, nano-length-scale, or its combination [21], or even at bulk length scale [22] the induction of porosity, whether at a micro-meter length scale or nanometer, length scale does allow an enhanced number of binding sites to encourage cell-proliferation. Nonetheless, a synergistic approach to consolidate these to enunciate amino acid interaction at molecular length scale, attain directional cell-growth in micro-textured surface and allow osseointegration at bulk length scale needs attention. Our research group has earlier elicited the control of bi-modal porosity via adopting different processing technique and utilization of porogen (pore forming agent) during processing of HA-based composites [23]. The bi-modal nature of porosity (i.e. sub-micrometer and micrometer sized pores) allows interaction of amino acids with enhanced interaction points, and an enhanced cellular growth is observed for the same [24].

It may also be noted that the macro porosity to the tune of 40-70% is mandated to permit vascularisation. In addition, the porosity size must be big enough (~ 100-250 μm). Further, micro-porosity similar to the size of human cell (~10s of μm) is required to permit directional growth of the cell. Also, it is observed by researchers that presence of nano-porosity (~nm size) permits an enhanced interaction of surface proteins with the cell surface at atomistic level. The protein interaction is originated at atomic length scale and allows sensing of 'surface-compatibility' of artificial implant material with the cells. Hence and amalgamation of nano-porosity (for molecular protein interaction), micro-porosity (for cell-directionality), and macro-porosity (for vascularisation) is needed for the bone-scaffolds.

ROLE OF ANTIOXIDANTS

Antioxidants, which are also present in fruits, assist in the expedited healing. Antioxidants take up the ROS and minimize the damage to the cell-walls. The aliovalent oxidation state of various cations allows trapping the ROS and restricting the damage it may cause to the living cells. During healing, the immune response triggers the release of ROS to clear out the debris and restrict infections. Without distinguishing between the anti-bacterial or site-cleaning process, ROS also becomes deleterious for human cells in the vicinity. The antioxidants assist in restricting ROS damage by combining with it and making it bearable for human cell. Just the larger size and membrane enclosed organelles in human cell permit sustenance while the anti-infection process is carried out by ROS. In summary, the M^{n+} oxidation state changes to M^{n+1} when combining with ROS, which then restricts the damage to the protective cell lipid bilayer. Typically, cerium oxide has been used as an antioxidant and has shown

to enhance the cell-proliferation process. Enhanced cellular affinity and growth in HA-CeO_2-Ag-CNT based samples [25], confirmed via absorbance is attributed to enhanced anti-oxidant capability (due to incorporation of CeO_2) that is elicited in scaled-up plasma sprayed coatings. Further, the balance of mechanical, biological and anti-bacterial properties must be sought to ensure enhanced service life of the femoral stems during THA [25].

ROLE OF EXTERNAL FIELDS

Both, magnetic [26,27,28] and electric fields [29,30] have been utilised to control the cell-fate processes. It is interesting to note that the ion-exchange and cell-permeation is controlled by the surface charges, which can be manipulated by the external fields. In addition, it is not only the magnitude, but also the pulsing (or frequency) that requires synchronised response from the cell to control the ion-permeation response. On one hand, where ferro-magnetic additives in HA can provide antibacterial efficacy supplemented with magnetic fields [26], on the other hand the ferro-magnetic responsive nature of cells [27 28] can be utilised in assisting osteogenesis with ferro-magnetic reinforcements. Similarly, the gene expression and cell-fate can also be tailored using complementing electric fields [29-33] or along with substrate conductivity [34-35] for obtaining enhanced cell-proliferation and directing the ensuing cellular-response forwards tissue engineering.

WETTING, PROTEIN ADSORPTION AND ADHESION OF BACTERIA/CELLS

A good scaffold material is also dictated by the wettability of the material as the subsequent protein adsorption is followed by cell-attachment and consequent cell growth. As an implant is inserted, protein attachment occurs almost immediately. Then, the nature of protein adhesion [36] on that substrate dictates what portion of that protein is available to attach (as ligand) with integrins (surface protein receptors of cells). Though cellular attachment is to be promoted, the 'friendliness' of the substrate may also allow adhesins (bacterial surface proteins) to initiate infection. Nonetheless, integrins allow attachment of cells by binding to ligands (e.g. fibronectin, collagen, laminin in bone cells) on an implanted surface. In order to observe role of these surface proteins, gene-expression may be undertaken to utilise these markers in linking it to bone growth. It may be noted that both, hydrophilic and hydrophobic surfaces may promote protein attachment. Earlier, cellular attachment and bacterial attachment events on substrates have been quantified by various researchers [36, 37]. Porosity has shown to encourage fibrous cell-ingrowth for additional mechanical interlocking, but it is the substrate chemistry that dominates in dictating if the protein is configured to allow a surface towards promoting cell-attachment and growth (i.e. Al_2O_3 bio-inert ceramic may not promote cell-growth, but bioactive HA promotes chemical binding with cells). As the attachment and adhesion events of cells can be qualitatively compared from centrifugation, or spinning disc or plate and wash assays, etc., whereas qualitative adhesion strength can be evaluated from optical tweezers, nano-scratching or atomic force microscopy, etc. Nonetheless, visualisation of the protein adhesion (or de-adhesion event of bacterial protein) requires molecular dynamics simulations.

The computational modelling is not only demanding, but also highly complex. First, the protein may have numerous stable confirmations in 3-D space, so selecting an appropriate profile may require multiple and repeated simulations. Second, only a portion of the protein (Fig. 2) may participate in the adhesion process, but with molecular weight of protein running in 100s of kilo-Daltons becomes highly computationally demanding to accommodate so many atoms. Moreover, the aqueous environment also changes the protein confirmation and requires appropriate stabilisation with the pH and salts. Then, an enough simulation space needs to be created to ensure that the protein is always under a water environment, and its stretching will always be accommodated in the simulation volume (and without affecting its mirror atom if it appears in the periodic boundary conditions). Nonetheless, visualization of these events may allow integration of the number of events (with multiple protein chains) with the magnitude of de-adhesion force can help comment on the strength of protein binding with various bio-surfaces.

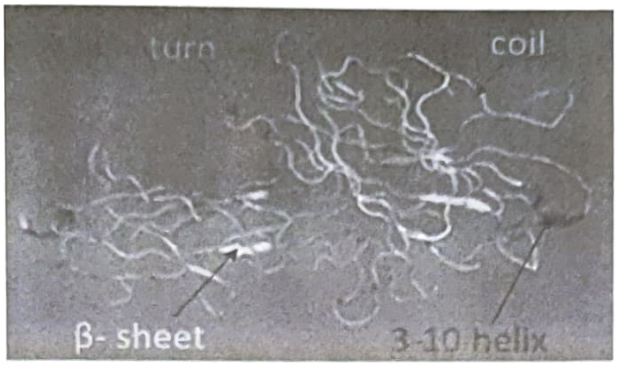

Fig. 2: A schematic showing portion of highly complex bacterial surface protein structure. It comprises of various helices and β-sheet structures and can undertake numerous 3-D conformations. (Courtesy: Mr. Arindam Raj, Yale University).

With advancements in the manufacturing technology, 3-D printing has emerged as a leading processing tool for constructing porous bone-scaffolds [38]. These constructs may be applied to cranial segments, skull, manibular portions, etc. The porosity design allows promoting enhanced capillarity/wetting along with enhanced surface area for biological/bioactive fixation. In addition, scaffold structures may be loaded with multifunctional additives during the 3-D printing process [39]. Further, the multi-functional scaffolds may restrict cancerous cell growth and provide therapeutic effects [39] and extend new opportunities in taking the bone-scaffolds as superior replacement to natural bone.

SUMMARY AND CONCLUSIONS

In summary, the approach of creating multifunction hydroxyapatite (HA) based scaffolds, Fig. 3, require appropriate combination of surface bioactivity, structural integrity, and porosity at multiple length scales. Further, antibacterial aspects may be incorporated with various additives such as Ag, ZnO, TiO_2, Fe_3O_4, or even anti-microbial peptides. The size and morphology of these additives may be highly important in promoting antibacterial effects. The healing events can be expedited with the addition of antioxidants (such as CeO_2) in order to curb the damage by reactive oxygen species. Damage tolerance of the HA-based composites may be promoted with addition of carbon nanotubes (CNTs), though metallic additives (such as Ag and Ti) may be more probable toughening agents. The presence of porosity at different length scales is mandated from promoting amino acid adhesion at molecular length scale to promoting vascularisation at macro-length scale. Functional gradation may also be envisaged to account for surface activity clubbed with bulk toughness of the HA-based bioactive composites. Visualisation of the protein attachment and bacterial de-adhesion events call for computational modelling approach, which can then be linked with its adhesion strength on various bio-substrates. Accounting for wettability aspects and protein adhesion, the cell-attachment must eventually ensure a successful osseointegration.

Fig. 3: The requirements of a multi-functional porous hydroxyapatite-based bone-scaffold.

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