Point-to-point Response to Examiners' Comments
(14AT91R14)
INDIAN INSTITUTE OF TECHNOLOGY KHARAGPUR
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Name of the Student: **Abir Dutta**

Title of the Thesis: **Design and Development of Lattice Structured Bone Analogue for Mandibular Reconstruction**

[Response]: The authors thankfully acknowledge the recommendations of the Examiners. The revised thesis and the point-to-point response to the Examiners' comments are hereby submitted.

Examiner (Foreign) 1

General Comments:

Strength of the thesis

Comment 1: This is an ambitious project covering broad range of multidisciplinary areas of mechanical engineering, materials fabrication, 3D printing, surface engineering, and biological characterisation, both *in vitro* and *in vivo*.

[Response] The author thankfully acknowledges the comments of the reviewer.

Comment 2: FE models of healthy and diseased mandibles were developed, considering all the teeth, periodontal ligament and thin soft fibrous tissue layers around the condyle. This has been applied to investigate the effects of odontogenic tumour size on load transfer across a mandible. The work is of publishable value as evidenced in a peer reviewed journal.

[Response] The author thankfully acknowledges the comments of the reviewer.

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Weakness of the thesis

Comment 3: FE analysis of lattice structures for bone scaffolds suitable for mandibular reconstruction was carried out with a set of limited design parameters. However, no validation was conducted on 3D printed Ti scaffolds. The designed optimal construct dimensions e.g. strut diameter and inter strut distance were not experimentally verified.

[Response] The author would like to clarify that the present study was primarily focussed on understanding the load transfer across a diseased and reconstructed mandible during a complete mastication cycle. The fabrication of the lattice structures was undertaken in order to gain insight into the feasibility of the designed pore architecture parameters of the structure to be manufactured using slurry based extrusion 3D printing. After successful fabrication, strut diameter and inter-strut distances of the sintered scaffolds were investigated using Scanning Electron Microscopy (SEM), which resulted in a conclusion that the process is capable to fabricate the scaffolds very close to the designed pore architecture parameters. However, it is essential to further investigate the dimensions of the sintered product rigorously to carry out the batch production of the scaffolds.

Comment 4: All FEA work was only carried out based on static loadings under a complete mastication cycle. No cyclic loading cases simulating repeated mastication were investigated.

[Response] The author thankfully acknowledges the comment of the reviewer. However, the present study was primarily focussed on load transfer across diseased and reconstructed mandibles and developing a numerical framework to evaluate the effective mechanical properties of the scaffolds. In this regard, quasi-static load cases of a complete mastication cycle was applied as loading conditions to all the FE models investigated in the study. Further investigations are warranted to understand the load transfer under cyclic loading conditions.

Comment 5: There was no consideration of design of other strut structure more suitable for osseointegration e.g. diamond or gyroid. Only 0°/90° Ti lattice structure

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was investigated which may not necessarily be the best structure for bone ingrowth and ongrowth.

[Response] The author thankfully acknowledges the comments of the reviewer. 0°/90° Ti lattice structures are one of the several lattice structured scaffolds, investigated for bone-graft analogue application. However, compared to the other scaffolds, such as diamond, gyroid etc., 0°/90° lattice structures are most suitable to fabricate using extrusion based 3D printing, along with the provision of enough room for tailoring of pore architecture parameters. That intrigued the author to investigate such a structure which allows both the flexibility to tailor the design parameters and ease of fabrication.

Comment 6: 3D printing of Ti scaffolds with extruded lattice structure and surface peptide modification demonstrated the good cell adhesion and viability. However, the long term osseointegration of scaffolds was not investigated.

[Response] The author thankfully acknowledges the comments of the reviewer. The experimental section of the study was devoted to gaining insight into the feasibility of fabrication of the 0°/90° scaffolds using extrusion based 3D printing and further understanding the influence of the surface modification methodology on the tissue integration *in vivo*. Further investigations including osteogenic differentiation of stem cells and large animal *in vivo* analysis are required to conclude and strengthen the outcome of the thesis.

Comment 7: There were a few typos e.g. p17, investing properties; p30, particle leech; p35, alongwith; p93, lesser ID and inappropriate use of technical terms, e.g. most material properties should be mechanical properties , to be precise and specific, because material properties could also include other physical and chemical properties.

[Response] The author would like to clarify that the typographical errors are rectified and the thesis has been revised accordingly.

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Questions to be asked during thesis defence

Question 1: What is the main advancement of this project in designing lattice scaffolds for mandibular reconstruction in comparison with other state of the art studies?

[Response] This study sought to explain the influence of odontogenic tumours on load transfer across the diseased mandibles using FE analysis, in order to further investigate on the design of customised Ti lattice structured bone graft analogue along with different types of reconstruction plates and screws, suitable for maxillofacial applications. Geometry and material properties were based on subject-specific CT-scan data. Static analysis considering multiple musculoskeletal load cases, representing a complete mastication cycle was considered as applied loading condition in the study.

Question 2: If the solid implants are reported to be prone to failure due to aseptic loosening and stress shielding, why did you still investigate solid Ti reconstruction plates in Chapter 4? How did it compare to the lattice reconstruction scaffolds?

[Response] The author would like to clarify that failure of maxillofacial reconstructions are reported to be influenced by combination of fixation screw types, design of the plates, lattice structured bone graft analogue, condition of the host bone tissue, and post-surgery physiological loading conditions. This led the author to investigate the influence of screw types on the load transfer across both the solid plate and plate with holes after reconstruction.

The lattice structured bone graft analogues were modelled as homogeneous solids having effective material properties of the scaffolds, as discussed in Chapter 3, using a novel numerical homogenization technique along with FE analysis. The influence of the load transfer through the scaffolds were evaluated as a part of the complete assembly modelled to represent the post-surgery reconstructed mandible.

Question 3: What are the limitations of the extrusion based 3D printing technique? How to control the dimensional accuracy for 3D printed Ti scaffolds? How to predict the shrinkage of the 3D printed green Ti scaffolds during high temperature sintering?

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[Response] The crucial part of printing with metal powders is to prepare it in such a form which would be able to overcome the yield stress of the nozzle and flow, as well as would sustain its circular shape after deposition on the printing bed. The metal powders are required to be processed to a homogenized dough by mixing with a polymer. The inclusion of the polymers also leads to several critical issues which play an important role in determining the precision of the output structure. The ratio between the powder and polymer should be optimized to achieve an appropriate green stage structure after printing. The struts would suffer from waviness or microcracks if the ratio is not at its optimum composition. Moreover, the metal powder should be handled within a closed environment, preferably under a glove-box or vacuum hood, to protect the surfaces of metal particles from getting oxidized. Careful attention is required to optimize the flow behaviour of the dough so that could overcome the yield stress of the internal wall of the nozzle. In this regard, the characterization of the rheological properties of the dough is necessary. However, there lies a limitation in the powder percentage in the composition. The range of maximum powder loading, which yielded to successful printing of circular fibers, was 80–85%. Low powder loading would result in sagging of the structures, and high powder loading would result in a high viscosity of the dough, which again adversely affect the printing parameters. The dough becomes highly viscous in cases of powder loading more than the allowed percentages, which loses its ability to flow through the nozzle. Inter-fiber contact is another issue which affects the integrity and mechanical strength of the printed structures. This issue is solved when the input parameters for slice thickness are prescribed. The optimum slice thickness should be 20% less than the nozzle diameter of the extrusion printer to achieve appropriate inter-connection between the fibers of two consecutive layers. However, the percentage of shrinkage, after drying in a vacuum oven and after the thermal treatment or sintering, should be considered while printing the green structures. Once the dough is prepared at its optimum composition, the pressure and printing speed are two major parameters to decide the successful completion of the printing process. The pressure should be optimized within such a range, so that the deposition of the dough through the circular nozzle remains intact. Higher pressure leads to more material deposition and lower pressure leads to discontinuous extrusion of the fibers. However, the printing speed is also associated complementarily with the pressure range. High pressure ranges require high printing speed, so that the fast-moving nozzle would drag the over-extruded fiber

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in its continuous circular form. Though, high speed and pressure combination can affect the integrity of the inter-fiber junction. In a nutshell, material processing (i.e., preparation of the dough, specifically for metal and ceramic printing), printing pressure and printing speed are the key parameters which decide the final form of an extrusion-printed structure.

Question 4: Why sintered Ti scaffolds at 1400 C? What would be the implication of sintering temperature to the physio mechanical properties of the 3D printed Ti scaffolds?

[Response] The green titanium scaffolds were fabricated by 3D printing of the Ti6Al4V powder loaded chitosan slurry. The dried scaffolds had loosely held Ti6Al4V powder interlocked by chitosan binder. The green scaffolds after heat treatment at 1400°C with dwelling time of 8 hours resulted in binder burnout followed by particle rearrangement, particle-particle contact formation, neck formation and growth, and mass transfer to form the granular structure associated with sintering. As a result, the sintered samples have significant increase in strength due to grain growth, pore elimination, and densification as compared to green samples.

Question 5: Apart from the surface modification with grafted osteogenic peptides, what are other factors that would affect the osseointegration of Ti scaffolds?

[Response] The following factors, amongst several others, would be crucial in determining the osseointegration capability of Ti scaffolds: pore size distribution, inter-connected pores, enhanced surface area, multiscale surface roughness, and appropriate protein adsorption.

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Examiner (Indian) 2

General Comments:

Strength of the thesis

This work is focused on designing and developing implants for segmental and complete mandibulectomy using FEA. Development of mandible models based on patient CT scans and designing appropriate implants to suit the defect is good. Moreover, the property assignment based on HU of CT scan is very new and logical. The use of lattice structures in the implants and its influence on the stress and strain in different parts of the mandible, implant, etc. is also very well thought. The experimental work on 3D of scaffolds, their surface modification followed by *in vitro* and *in vivo* trials improved the quality of the work significantly. The overall scope and approach considered were logical. The outcome of the investigation is extremely important and provides essential understanding/information related to the design and manufacturing of long-lasting implants for segmental or complete mandibulectomy.

[Response] The author thankfully acknowledges the comments of the reviewer.

Weakness of the thesis

1. In each chapter, the limitations and weakness of current study have been indicated. However, no suggestions were included to address these limitations.

[Response] The author would like to clarify that each of those limitations carry the potential to direct and undertake multidirectional research methodologies and investigations; therefore general limitations, explaining the possible research gap and further avenue to refine the results were stated within each of the chapters of the thesis. Furthermore, broader discussion on future scopes were discussed in the 'Chapter 6: Conclusions' of the thesis.

2. At some places additional discussion is required to explain the results and other places no discussion was present.

[Response] The authors would like to clarify that the thesis has been revised accordingly.

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3. The *in vitro* and *in vivo* data is encouraging, but as indicated in the thesis, osteogenic potential of the scaffolds would have considered.

[Response] The author thankfully acknowledges the comments of the reviewer. The experimental section of the study was devoted on gaining insight into the feasibility of fabrication of the 0°/90° scaffolds using extrusion based 3D printing and further understanding the influence of the surface modification methodology on the tissue integration *in vivo*. Further investigations including osteogenic differentiation of stem cells and large animal *in vivo* analysis are required to conclude and strengthen the preliminary outcome of the thesis.

4. The text related to materials and methods and other sections appear to be similar in different chapters. Please revise the text or refer to earlier sections as appropriate to avoid repetition.

[Response] The author would like to clarify that the texts in the thesis have been revised accordingly.

I recommend *"The thesis be accepted after minor modifications/ revisions as suggested. After modification, the thesis need not be referred to me again."* There is no need to perform any additional experiments. However, the thesis should be revised considering the following comments.

[Response] The authors thankfully acknowledge the recommendation of the reviewer. Point-to-point response to the reviewer's comments is presented herewith.

Comments

Comment 1: I noticed some typographical and language errors (Pages xiii, 10, 22, 56, 84, 152, 155, etc.) and suggest thorough proof reading of the thesis.

[Response] The author would like to clarify that the typographical errors are checked and the thesis has been revised accordingly.

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Comment 2: The text related to 'materials and methods' and other sections appear to be similar in different chapters. Please revise the text or refer to earlier sections, as appropriate, to avoid repetition.

[Response] The author would like to clarify that the thesis has been revised accordingly.

Comment 3: At some places additional discussion is required to explain the results and other places no discussion was present.

[Response] The authors would like to clarify that the thesis has been revised accordingly.

Comment 4: In each chapter, the limitations of current study have been indicated. It would be good to suggest/propose how these can be eliminated in future studies.

[Response] The author thankfully acknowledges the suggestion of the reviewer. However the author would like to clarify, as each of the limitations might have multiple possibilities and with attempt of experimentation only, it would be better understood the most suitable approach for the solution. Therefore we have not highlighted on the specific proposals to deal with the specific problems.

Comment 5: The results of load case 3 have been included and discussed in detail. It would be better to include the results of other load cases as Appendix.

[Response] The author thankfully acknowledges the comments of the reviewer. However, the author would like to clarify that, if all the load cases included, there would be enormous number of diagrams exceeding the 120 numbers (current image counts of the thesis: 44; if images corresponding to all the load cases and both sides of the mandible, are to be included, approximately additional 85 images are to be included, leading to a total 129 numbers of images); therefore for better representation, only the relevant and most important diagrams are included in the thesis.

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Abstract

Comment 6: A summary statement combining both studies (FEA and 3D printing of final implants) at the end of the abstract is suggested. Also include some experimental data in the abstract.

[Response]The abstract has been revised according to the reviewer’s suggestion. The following sentence has been included within the Abstract of the thesis:
“The study has established that the FE based design of the scaffolds were successfully fabricated using 3D printing and showed enhanced tissue integration abilities while grafted with osteogenic peptides on their surfaces.”

Chapter 1

Comment 7: Page 6. Section 1.3: The role of patient specific implants in improving the clinical outcome of implants should be clearly highlighted. In addition to the points discussed in the thesis, it is suggested to add details on the variations in tumor size, location and anatomy of the patients, etc.

[Response] The author thankfully acknowledges the comments of the reviewer. However the author would like to clarify that the texts in Page 6 are a part of the genesis of the customized implants i.e. from usage of grafts to several underlying issues of the grafts as well as implants, and finally leading to the patient-specific design and development (described in Page 7 of the thesis) of implants. Pathology of the tumours was not the primary focus of this study. Therefore, relevant references were included alongside the text indicating resources for specific details and literature for tumour pathology.

Comment 8: Page 9, Last paragraph: Revise the text, as the low fracture toughness and fatigue resistance is not applicable to Ti alloys currently in use. They have sufficiently high dynamic properties to suit functional requirements of wide variety of load bearing implants.

[Response] The paragraph has been revised according to the reviewer’s suggestion.

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Comment 9: Page 10. 1st paragraph: 'Osteolysis’ it is related to wear debris induced bone loss not due to stiffness mismatch!

[Response] The author would like to clarify that the paragraph has been revised accordingly.

Comment 10: Page10: Last paragraph: Some part of the text is smaller than other. Please check the font size.

[Response] The author would like to clarify that the paragraph has been revised accordingly.

Comment 11: Page 11, Last paragraph: “The regularised pore geometry, popularly known as lattice structures are preferred compared to random pores for orthopaedic implants” Explain why?

[Response] The author would like to clarify that despite preferences of natural tissues towards random pore architectures[1], regular pore architecture[2] are preferred owing to its easier adaptation in terms of fabrication methodology and evaluation and assignment of effective material properties to the simulation environments.

Comment 12: Page 14: The first two stages of mastication cycle are not universal for all foods. You may add additional text on this.

[Response] The author thankfully acknowledges the comment of the reviewer. However, the author would like to clarify that influence of types of the foods was not the primary focus of this study, therefore, was not included in any of the developed FE models presented in the thesis. Thus, in a more general perspective, all the loading cases were discussed to investigate the load transfer across the mandible models.

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Comment 13: Pages 57: The FE models of diseased jaw were derived from CT scans of patients with different age to study the influence of tumor size. It is important discuss how this can influence the results. Age related changes in the bone properties and life style (especially the diet) of the patient.

[Response] The author thankfully acknowledges the comments of the reviewer. Age and metabolic activity of a patient indeed are known to potentially influence the overall effective material properties of the human hard tissues. However, this aspect is not the focus of the current study. The author would like to clarify that the CT-scan datasets were acquired for the following specific objectives:

(1) Development of solid models of the tumour affected mandibles,
(2) Identifying the heterogeneous material properties of the diseased cancellous bones using a CT-scan data based numerical algorithm based on a density-modulus relationship.

Further investigations are warranted in order to investigate the influence of age, ethnic origin, metabolic activity and pathology of tumour on the load transfer across a diseased mandible during physiological loading conditions.

Comment 14: Page 58: “The density-modulus relationship predicted the average Young's modulus of 943 MPa and 623 MPa for the cancellous bone....” Please specify the region of the bone in the respective mandibles (is it very close to the tumor?)

[Response] The author would like to clarify that the “*predicted average Young's modulus of 943 MPa and 623 MPa for the cancellous bone*” were values of the Young’s modulus of the respective diseased cancellous bones, averaged over the corresponding heterogeneous Young’s modulus of all the volume elements. So, the averaged Young’s modulus values can not be specified or interpreted as a single homogeneous region.

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Comment 15: Page 60: "Hence, it was assumed that the overall material properties of the cortical bone of the diseased jaw were also reduced by the same percentage as it was for the cancellous bones." what decides the reduction in modulus? If it is the density/porosity then from the HU the density and hence the modulus of cortical bone can also be estimated.

[Response] The Young's modulus of the healthy mandibular cancellous bone was determined using the density-modulus power law relationship, $E = 2017.3\ \rho^{2.46}$, reported by Dalstra et al.[3].This particular density-modulus relationship predicted an average Young's modulus of 1093 MPa for the healthy cancellous bone, which was close to 960 MPa reported earlier for the mandible [4]. The density-modulus relationship predicted the average Young's modulus of 943 MPa and 623 MPa for the cancellous bone regions, for the models ODT1 and ODT2, respectively. The Poisson's ratio was assumed to be 0.3 for both models. It was observed that the Young's modulus values were reduced by 13.72% and 43% for the cancellous bones of models ODT1 and ODT2, respectively, in comparison to the healthy cancellous bone (Model A). A similar approach to determine the cortical bone material properties could not be undertaken due to the problem of partial volume effect in the cortical bone regions of the FE model. Hence, it was assumed that the overall material properties of the cortical bone of the diseased jaw were also reduced by the same percentage as it was for the cancellous bones.

Comment 16: Page 60: Where are the details of load cases?

[Response] The author would like to clarify that "*the details of the muscle forces and the prescribed boundary conditions during a mastication cycle are presented in Table 1.4 in Chapter 1 of this thesis*"[4, 5].

Comment 17: Fig. 2.4: The results shown are for a first cycle? Do you expect any changes in the stress with number of cycles? How that will influence your outcome.

[Response] The author would like to clarify that for a first complete mastication cycle, consisting of six load cases, equivalent stress distributions across a healthy mandible were presented in Figure 2.4 in the thesis.

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The author would like to clarify that there might be potential influence on the stress distributions under repetitive mastication cycles. However, further investigations are required to conclude on the influence of repetitive loading cycles on the load transfer across the mandibles.

Comment 18: Fig. 2.5: One molar tooth is missing in ODTI and how this can influence on the results?

[Response] The author agrees with the examiner that one molar tooth in the CT-scan data was missing (absent). According to the clinician's perspective, during molar bite, first and second molar teeth are primarily active during the occlusion. The nodes on the occlusal surface of the right molars were restricted from movement along the transverse direction and the muscle forces were applied on patches on the surface of the cortical bone during the right molar bite.

The focus of the study was to investigate the load transfer across the diseased mandibles with different degrees of odontogenic tumours during a chewing cycle. The total load, which is normally applied on three molar teeth, is now applied through the available two molar teeth in the FE model (based on patient-specific CT-scan data). Hence, the total load transferred on the mandible remains unaltered.

Comment 19: "High principal compressive stresses (20-50 MPa) were generated in the regions under canine of the working side of the mandible in Model ODT2 as compared to those in Model A and ODTI." Please discuss /explain the reason behind this observation.

[Response] The regions under canine of the working side of the mandible in Model ODT2 as compared to those in Model A and ODTI were observed to be under high principal compressive stresses (20-50 MPa). This is due to the differences in the structural geometry of the mandible for different cases (healthy mandible, mandible with small sized tumour and mandible with large sized tumour). For the same loading and constraint conditions, stress distributions are known to vary considerably with change in structural geometry and inclusion of geometric discontinuities.

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Comment 20: Page 70: Is the tumor studied in this work is a solid mass? Not visible as solid in the CT or other images.

[Response] The author would like to clarify that the tumours were not modelled like a solid mass. The tumour affected mandibles were segmented into teeth, cortical and cancellous bone volumes, based on the grayscale (in Hounsfield Unit) values using MIMICS (MIMICS, Materialise, Leuven, Belgium). In case of the Model ODT2, the cortical and cancellous bones were completely perforated because of the presence of tumour. The cortical bone of the Model ODT1 was not at all perforated, but the tumour has affected the geometry of the cortical bone of the body region of the mandible (Figure 2.3).

Comment 21: Page 71: "Maximum principal tensile stresses (~ 49 MPa) was observed in the Model ODT2," Why? The stress was only 21 MPa for healthy bone in spite of its high modulus compared to ODT2?

[Response] The author would like to clarify this is due to the differences in the structural geometry of the mandible for different cases (healthy mandible, mandible with small sized tumour and mandible with large sized tumour). For the same loading and constraint conditions, stress distributions are known to vary considerably with change in structural geometry and / or inclusion of geometric discontinuities.

Comment 22: Page 71: "It is evident from the tensile stress distributions that the large-sized tumor was responsible for stress concentration and.." is it due to modulus change or loss of bone density or porosity or change in the geometry of the bone? Add additional discussion on this.

[Response] The authors would like to clarify that the stresses around the tumour were found to be more concentrated in Model ODT2 owing to the presence of the tumour perforating the body region of the mandible similar to a hole. Primarily, the elevated stress concentrations might have been influenced by differences in the structural geometry of the mandible models and the data were acquired from the living individuals. However, further investigations are required to identify the influence of

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the bone density and porosity of the diseased bone. The texts in the thesis has been revised accordingly.

Comment 23: Page 72: “The decrease in Young's modulus of the cortical bones of the diseased mandibles led to a weakening of the bone structure, eventually making the structure vulnerable to high strains “. Can this lead to simultaneous increase in the stress as mentioned earlier? Also, “comparison of maximum tensile strains for Model ODTI and ODT2 indicated that the tensile strain values increased by 96% with a reduction of Young's modulus from 14% to 43%.” What about the maximum stress on these models as discussed earlier? Please examine these points carefully and revise the text accordingly.

[Response] The author would like to clarify that stress is more primarily influenced by the geometry of the structure, whereas strains are influenced corresponding to the material properties of the structure. It warrants further investigation to conclude whether an increase in strains could lead to increase in stresses in such a complicated structure of mandible.

The maximum stresses of the tumour affected models were discussed in the last paragraph of page 71 of the thesis.

Chapter 3

Comment 24: Page 78, 2nd paragraph: The influence of 0°/90° strut orientations has been discussed, but its influence on other properties such as modulus, biological properties can be included.

[Response] The author would like to clarify that the primary focus of the study undertaken in Chapter 3 in the thesis, was to develop a novel FE based numerical homogenization technique to evaluate homogeneous orthotropic material properties of the 0°/90° lattice structures with six different combination of pore architecture parameters of strut diameter and inter-strut distance. Therefore, within the scope of the current study (Chapter 3) the influence of strut diameters, inter-strut distances of 0°/90° Ti lattice structures was discussed.

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Comment 25: Page 78, Last paragraph: It is appropriate to clearly indicate why other unit cell types (BCC, Diamond, TPMS) were not considered.

[Response] The author would like to clarify that there are numerous numbers of research articles [2, 6-14] considering different types of lattice structured scaffolds such as BCC, diamond, TPMS etc. However, we had a broader aim to recreate the designed scaffolds using extrusion based 3D printing of polymer based Ti slurry. In this regard, within the scope of this chapter we discussed only 0°/90° scaffolds.

Comment 26: Page 85, Last paragraph: The text appears to be similar to the one in Chapter 2 and therefore may be revised or refer the earlier text.

[Response] The author would like to clarify that the CT-scan dataset used to develop the solid model of homogeneous lattice structured complete mandibular construct (CMC) was unique and completely different from the previous chapter. Despite following the same methodology, as the CT-scan dataset was different, the author had mentioned the methodology to enhance the article's clarity to the reader.

Comment 27: Page 86: "The mastication cycle was divided into six load cases, i.e., incisive, intercuspal position, right molar bite, left molar bite, right group function and left group function". Please add these details in the Chapter 2. I was looking for this clarification in the Chapter 2, as I could not understand what the "load cases" mean.

[Response] The author would like to clarify that "*the details of the muscle forces and the prescribed boundary conditions during a mastication cycle are presented in Table 1.4 in Chapter 1 of this thesis*".

Comment 28: Fig. 3.5: What is the difference between intact and CMC model? The latter model contains lattice structure? If so sectional views may be included and appropriate text should be included in the text and figure caption.

[Response] The intact model was assigned with homogeneous orthotropic material properties of healthy mandible, whereas the CMC models (Model A – F; Chapter 3) were assigned with homogeneous orthotropic material properties of respective 0°/90°

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lattice structures. The main focus of the chapter was to develop a numerical framework to obtain the homogenized orthotropic material properties of the Ti-scaffolds using microscale models of 0°/90° lattice structures, so that the calculated effective orthotropic material properties to be assigned to the respective homogeneous macroscale lattice structured CMC models. Please see section 3.2.3 ‘Macroscale FE models of intact and Complete Mandibular Constructs’, pages 84-86 in the Chapter 3 of the thesis.

Comment 29: Page 88, Table 3.3: Please explain and add details on how these properties were determined/ estimated.

[Response] The author would like to clarify that the details of the procedure to calculate the results in Table 3.3 were explained in the section “3.2.1 Numerical homogenization technique for scaffolds: microscale FE models”, page 80 in the Chapter 3 of the thesis.

Comment 30: Page 89, 2nd Paragraph: “The maximum principal stress of 34.54 MPa, was generated in the intact mandible.” Compare these results with Chapter 2 results and discuss. “However, the overall stress distribution (tensile and compressive) in the CMC models (30-32 MPa) were found to be very well compared with those of an intact mandible (34.54 MPa)” why? If the CMC are porous or contain lattice structure - please refer to point 27 above.

[Response] The author would like to clarify that the geometry of the intact mandible model in Chapter 3 is different from that in Chapter 2. In this regard, qualitative comparisons of stresses at comparable locations with the data published by Korioth et al. [4, 15]were discussed to validate the FE model. The geometry of the intact mandible model and the lattice structured homogeneous CMC models was no different to each other. This might have been the reason behind the observation of similar state of stresses across the intact and CMC models. However, owing to the difference in homogeneous orthotropic material properties between the intact and CMC models, state of strains were investigated and distinct differences were observed.

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Comment 31: Page 92: Please explain and add discussion on "It was observed that Model 'E' and 'F' exhibited principal strain distributions very similar to the intact mandible owing to the similarity between the material properties of the two scaffolds and the intact mandible."

[Response] The author would like to clarify that it was observed from Figure 3.6, that the Models E and F exhibited principal strain distributions similar to the intact mandible model. Close similarity was observed between the homogeneous orthotropic material properties reported for healthy mandible (Tables 1.5 and 1.6, Chapter 1, page 18 of the thesis) and the effective orthotropic material properties of the lattice structures Models E and F (Table 3.3, Chapter 3, page 88). The text has been revised accordingly.

Comment 32: Page 93, Last paragraph: Please measure/estimate the total porosity and correlate with mechanical properties. You can also compare the porosity with natural tissues as well. Further, consider other properties, in addition to weight, during selection of appropriate scaffold.

[Response] The author thankfully acknowledges the comments of the reviewer. The Table 3.3 has been revised accordingly. The last paragraph of the section 3.4 (page 93 of the thesis) has been revised accordingly.

Comment 33: Page 94: Please discuss these conclusions in detail - total porosity, mechanical properties, etc. and their correlation with natural tissue, etc.

[Response] The author would like to clarify that porosities of the scaffolds were calculated (Table 3.3) and discussed in the 'section 3.4 Discussion' of the Chapter 3 in the thesis. The main focus of this study (Chapter 3) was to develop a numerical framework to evaluate the homogeneous orthotropic material properties of the 0°/90° Ti lattice structures. Moreover, based on the macroscale analysis of six CMC models under a complete chewing cycle, state of stresses and strains,overall porosity, and weight of the CMC models were considered to choose suitable pore-architecture parameters for the design of Ti-CMC. However, further investigations using microscale and macroscale models of 0°/90° lattice structures are warranted in order

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to gain insight into the influence of porosity on the load transfer during a complete mastication cycle.

Chapter 4

Comment 34: Page 104, 2nd paragraph: The text appears to be similar to the one in Chapter 2 and therefore may be revised or refer the earlier text.

[Response] The author thankfully acknowledges the comment of the reviewer. The texts have been revised accordingly.

Comment 35: Page 106, 2nd paragraph: Please add details and images of lattice structured bone graft showing internal structure.

[Response] The author would like to clarify that the lattice structured bone graft was modelled as a homogeneous solid structure of the exact shape of the bone graft (Figure 4.1c) following the methodology and results obtained in Chapter 3 in the thesis. The sole purpose of the study was not to use the computationally expensive FE models of 0°/90° Ti lattice structures having the struts within the model; therefore homogenised material properties of the scaffolds were calculated in the Chapter 3 of the thesis, and the same properties of the scaffold having 0.6 mm strut-diameter with 0.5 mm inter-strut distance (Model F) were assigned to the homogeneous bone graft in the Chapter 4.

Comment 36: Page 107, Last paragraph: A pictorial or schematic delineating the contacts and boundary conditions would improve the clarity.

[Response] The author thankfully acknowledges the recommendation of the reviewer. The details of the boundary conditions has been included in the Figure 4.1 (page 105 in the thesis).

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Comment 37: Page 111: Please explain and discuss why the stresses were high for bicortical screws? Also, explain why the stresses are less for bicortical in anterior buccal areas for reconstruction plates? Which is opposite of stresses observed in implanted mandibles (Fig. 4.3).

[Response] The author would like to clarify that within the current scope of the study, the geometry of the assembly of the reconstructed mandibles were different to each other based on fixation screw placements. So, it can be explained that primarily due to difference in geometry the stresses were found higher in the bicortical screws as well as anterior buccal areas of the reconstruction plates. However, further investigation using microscale models of screws and plates in a reconstructed mandible is required to understand the influence on the load transfer during physiological loading conditions.

Comment 38: Page 112: Fig. 4.4 not discussed and came before the discussion related to Fig. 4.5 and 4.6.

[Response] The author would like to clarify that the Figure 4.4 was discussed later to Figure 4.5 and Figure 4.6, as both the latter figures were based on stresses of plates and screws i.e. components of the complete assembly of post-surgery reconstructed mandible. However, owing to discuss the influence on complete reconstructed assembly, the Figure 4.4 appeared within the figures' list before the component specific results described in Figures 4.5 and 4.6. In the discussion section, the stress distributions across the FE models were discussed before moving to the discussion of strains.

Comment 39: Page 117: "High tensile stresses around the anterior buccal areas, from 'body' towards symphysis regions were generated in cases of mandibles implanted with Rp2 plate, as compared to stresses generated in comparable locations of the mandibles implanted with Rpl plate.'' Discuss the implications of these outcomes on the performance of the implants.

[Response] The author would like to clarify that the study (Chapter 4) was a preliminary report and first-of-its-kind investigation on the influence of types of

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fixation screws and plates across a reconstructed mandible considering the bone graft analogue, dental screws and crowns. Based on the results of the study, suitable combinations of fixation screw and type of plates were suggested in conclusion. Moreover, it was observed that none of the models reached the maximum yield point of Ti (900 MPa), therefore suggesting the assembly would not fail under the considered physiological load case (right molar bite). Further numerical and experimental investigations are warranted in order to conclude on the performance of the implants. Therefore, within the scope of the current study, following conclusions were drawn:

(1) Tumour affected mandibles after resection, may be reconstructed using solid Ti reconstruction plate and Ti bicortical screws, along with homogenized Ti bone graft analogue, dental roots and Zirconia crowns.

(2) Monocortical screws may be suitable for patient-specific Ti reconstruction plate with holes for reconstruction of tumour affected mandible.

However, the choice of the combination of types of plates (solid plate and plate with holes) with screws for fixation (monocortical and bicortical) may be assessed based on the condition of the host bone and spread of the tumour.

Comment 40: Page 117:"A possible explanation to this might be the presence of holes in Rp2 plate which generated lower stresses compared to plate Rp1" Explain how holes can contribute towards this? What changes you expect in the modulus of plates with holes? How these holes can accommodate the strains by localized deformation around these holes?

[Response] The author would like to clarify that, this is due to the differences in the structural geometry of the mandible for different cases (healthy mandible, mandible with small sized tumour and mandible with large sized tumour). For the same loading and constraint conditions, stress distributions are known to vary considerably with change in structural geometry and / or inclusion of geometric discontinuities.

The author would also like to clarify that, owing to presence of the holes, the geometry of the solid plate changes. The structure of the plate with holes offers less resistance (lower stiffness) to the load transfer across it, therefore less stresses were generated in case of the plate with holes. Further investigations are required to

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investigate the influence of the holes on the effective elastic modulus of the plates and strain distributions across the plates.

Comment 41: Page 117: "...an overall increase in maximum principal tensile strains were observed in implanted mandibles with plate Rp2 as.." what does this mean ? Better load transfer across the implant and mandible! Add discussion in these lines.

[Response] The author would like to clarify that presence of holes in the patient-specific Ti reconstruction plate, Rp2, was also accommodated with a compromise on the mass-loss as compared to the solid Ti plate (Rp1). In this regard, an overall increase in maximum principal tensile strains was observed in implanted mandibles with plate Rp2 as compared to those implanted with plate Rp1. The influence of the presence of holes on the maximum principal strains during the load transfer was discussed in this paragraph. Comparative statements were not given in this regard, as within the scope of this study, it could not be concluded as 'better' or 'best' in terms of load transfer across the reconstructed mandible.

Comment 42: Page 117. Last paragraph: Here again the results must follow a good discussion, which is absent.

[Response] The author would like to clarify that state of strains across the model consisting of Ti plates and screws were presented in this paragraph for brevity. However, the plates and screws were assigned with isotropic material properties of Ti (metal). In that regard, following the ductile failure theory for metallic components, the state of stresses of plates and screws were more important as compared to the state of strains.

Chapter 5

Comment 43: Page 132: Details of ethical approvals for *in vivo* trials should be included.

[Response] The author thankfully acknowledges the comment of the reviewer and the 'section 5.2.3.6 In vivo implantation study' in Chapter 5 of the thesis has been revised accordingly.

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Comment 44: Page 136. Fig. 5.5: Add results of feed stock powder also. High oxygen concentration and formation of intermetallics are detrimental to the properties. Further, the intermetallic formation in this alloy is unusual. Careful examination is required.

[Response] The author would like to clarify that the results of the phase analysis of the sintered Ti scaffolds were compared with the published data for the same batch of feedstock Ti powder by Kapat et al.[16] The results of the phase analysis undertaken in the current study was found to be in close agreement with the data published by Kapat et al.[16] Further detailed investigation regarding omission of the unwanted intermetallic phases from the sintered Ti scaffolds, is necessary prior to moving towards batch production of the scaffolds. However, within the scope of the current study, after successful fabrication of the scaffolds with designed pore architecture parameters, the results of the phase analysis provided confidence on undertaking further investigations of the sintered Ti scaffolds.

Comment 45: Page 137, Phase detection: Unusual intermetallics were observed in sintered samples. Detailed high magnification microstructural analysis can be performed to confirm the same in addition to comparison with feedstock Ti6AL4V alloy powder data.

[Response] The author would like to clarify that the 'section 5.2.5.3 Phase detection' in Chapter 5 of the thesis has been revised according to the suggestion of the reviewer.

Comment 46: Page 138. Fig. 5.6: standard microstructural analysis is required to confirm the absence of defects (pores, cracks, etc.) and microstructural abnormality.

[Response] The author would like to clarify that the primary focus of the study was to investigate the feasibility of the manufacturing of the 0°/90° Ti scaffolds using extrusion based 3D printing. Non-destructive evaluation of the sintered scaffolds using micro-CT preliminarily provided confidence on the capability of the extrusion based 3D printing on producing the scaffolds close to the designed dimensions.

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Further investigations are required before moving to the batch production of the scaffolds.

Comment 47: Page 138: "Porosity of these lattice structures were defined by pore architecture parameters, i.e., strut-diameter and inter-strut distance." where is the data? The density of struts or porosity inside the strut should be determined.

[Response] The author would like to clarify that the design of the 0°/90° scaffolds was based on two pore architecture parameters, strut diameter and inter strut distance, described in detail in Chapter 3 of the thesis. So, within the scope of this study, the primary focus was to investigate the feasibility of the 3D extrusion printing to fabricate the scaffolds close to the designed pore architecture parameters. Further investigations are required in order to identify the influence of the structural integrity of the struts on the effective material properties of the scaffolds.

Comment 48: Page 138, Last paragraph: Please correlate the mechanical properties of these scaffolds with their porosity, natural tissue and literature data. Also the ISO13314 is for porous and cellular materials not for AM metallic scaffolds.

[Response] The author would like to clarify that discussion of the homogeneous effective orthotropic elastic modulus of the0°/90° Ti lattice structures based on porosity and comparison with the natural tissue data was described in the Chapter 3 of the thesis. Within the limited scope of this study, effective Young's modulus was calculated along the transverse direction of the 0°/90° Ti lattice structures.

The author thankfully acknowledges the comment of the reviewer on following the ISO 13314 standard for material testing. However, the author would like to clarify that "ISO 13314: Mechanical testing of metals- Ductility testing- Compression test for porous and cellular metals" was considered for the sintered0°/90° Ti scaffolds (0.6 mm strut diameter having 0.5 mm inter-strut distance having porosity of 54.8%) owing to its porosity being > 50%.

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Comment 49: Fig. 5.10: Considering the formation of intermetallics and high oxygen concentration it would be better to comment/compare MTT/ cytotoxicity of present scaffolds with standard Ti6Al4V alloy.

[Response] The author would like to clarify that within the scope of the current study, the MTT/ cytotoxicity of the pristine sintered scaffolds was investigated with blank cell culture wells as control. In the next phase of the study, the surface modified sintered Ti scaffolds were investigated for cytotoxicity using human amniotic derived stem cells, having pristine Ti scaffolds as control. However, considering the formation of intermetallics, further investigations are required to understand the long term influence of the scaffolds *in vitro* and *in vivo*.

Chapter 6

Comment 50: Page 153, Last paragraph: Properties were assigned (based on lattice structure) to the bone graft analogue, but the physical model should also represent the porous structure - I could not see any figure to demonstrate this.

[Response] The author would like to clarify that the section has been revised accordingly, referring to the physical model of the 0°/90° Ti lattice structures and evaluated homogeneous orthotropic material properties in Chapter 3 of the thesis.

Comment 51: Page 154: "Mechanical properties of the sintered Ti scaffolds were observed to be close to those of human bones." How these experimental properties correlate with homogenized properties used for mandible constructs in the earlier chapter?

[Response] The author would like to clarify that within the scope of the current study, the effective Young's modulus (18.8 GPa) and the compressive strength (216 MPa) along the transverse direction of the of the sintered 0°/90° scaffolds (0.6 mm strut diameter having 0.5 mm inter-strut distance) were found to be close to those of human bones [7]. The orthotropic homogeneous properties of the scaffolds were calculated using a FE based numerical homogenization technique described in Chapter 3 in the thesis. In an ideal way, the orthotropic elastic material properties of the sintered fabricated scaffolds i.e. compression testing of the scaffold samples along three

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different directions, should be undertaken to verify the FE based homogenization technique in predicting the effective homogeneous material properties of the 0°/90° Ti scaffolds. However, within the scope of our current study effective Young's modulus along the transverse direction of the 0°/90° scaffolds were undertaken and found to be in close agreement with the predicted values. However, despite several literature referring the capability of the technique in prediction of the effective elastic material properties of the scaffolds, further investigations are required in order to provide confidence on the prediction of orthotropic homogeneous material properties of the 0°/90° Ti scaffolds.

Comment 52: Page 155, 2nd Paragraph: "Further study with more detailed load cases, and incorporating cyclic loading cases are necessary to gain more insight into the stress, strain related failure mechanisms." What about number of mastication cycles and food mechanical properties.

[Response] The author would like to clarify that further investigations are required to understand the influence of repetition of mastication cycles and food mechanical properties on the load transfer across healthy and reconstructed mandibles.

Comment 53: Page 155: Elimination of intermetallic and oxygen pick up is also very important.

[Response] The author thankfully acknowledges the comment of the reviewer. The section 6.3 of Chapter 6 in the thesis has been revised accordingly.

Questions to be asked to the candidate at the time of viva-voce examination

Question 1: "The lack of a natural state of stress due to normal physiological conditions induces bone resorption around the implant leading to osteolysis, implant loosening, and eventual failure". Why?

[Response] The bone is very adaptive to the nature of physiological loading. According to the mechanostat theory, the lack of natural state of stresses leads to bone

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resorption, further leading to underlying issues like implant loosening and eventual failure.

Question 2: What is the influence of stress distribution of bone on its remodelling and how bone cancer patient bone will response to this compared to normal patients?

[Response] The author thankfully acknowledges the potential behind the query of the reviewer. Material properties of the bones of the cancer patients are subjected to high inter-patient variability. Moreover, the geometry of the bones and underlying medication history of the patient at the time of bone cancer diagnosis also differ patient-wise. In that regard, without a detailed numerical and clinical investigation, it would be very premature to comment on the influence of stress distribution of bone on its remodelling for a bone cancer patient.

Question 3: Explain why lattice structures are preferred over random porosity for any engineering applications. Give one specific mechanical property that can be improved with lattice structures compared to random porosity sample with identical pore volume and size.

[Response] The load distribution in regular structures is much more predictable compared to the random pore structures. Moreover, owing to the dendritic nature of the structure of the random pore metal structures, those are not very easy to manufacture in terms of structural strength and support. The load carrying capacity of the regular lattice structured scaffolds are reported to be higher compared to those of the porous structures.

Question 4: Do you expect any changes in the stress with number of mastication cycles? How that will influence your outcome?

[Response] Based on the literature, it might be expected that the structure (bone in this case) would likely to reach the endurance limit at a stress level much lesser than its yield limit, under cyclic or repetitive loading.

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Question 5: Your results show high stresses during right molar biting in a healthy mandible, why?

[Response] The right molar bite consists of maximum amount of forces exerted by the muscles on the mandible during the load transfer. Moreover, the combination of the muscle orientation and the involvement of molar teeth, are also responsible to develop higher stresses during right molar bite.

Question 6: An increase in the tumor size decreased the elastic modulus of the diseased mandibles and resulted in elevated stresses and strains at comparable locations of healthy mandibles, why? (How decrease in the modulus can lead to high stresses and strains?)

[Response] An increase in the tumour size influenced the state of stresses and strains during the load transfer under physiological loading condition. However, it is to be noted that stress is to be influenced by geometry of the structure whereas strains are directly influenced by the modulus of the structure. In our case, the stresses are primarily varying based on the variation in the geometry as each of the geometry of the mandible is unique and patient-specific. However, reduction of the modulus influenced an increase in the strains in the models.

Question 7: What is the rationale behind studying the plates with holes for mandible reconstruction?

[Response] Within the scope of the current study, the rationale behind the studying the plate with holes for mandible reconstruction was to design a durable patient-specific reconstruction plate, which would also support the flushing of the structure with nutrients and saline during the surgery. However, further investigations are required to finalise the designs for further clinical assessments.

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Question 8: Cancer affected bones are not good candidates for modular implants, why?

[Response] Modular implants are now known for their fretting and wear issues *in vivo*. Cancer affected bones are already mutated by unknown functionalities. Further introducing the risk of metallic wear debris within the cancer affected bones must be avoided.

Question 9: What are the challenges of 3DP technique used in this work? (Supports, overhand features, etc.)

[Response] The crucial part of printing with metal powders is to prepare it in such a form which would be able to overcome the yield stress of the nozzle and flow, as well as would sustain its circular shape after deposition on the printing bed. The ratio between the powder and polymer should be optimized to achieve an appropriate green stage structure after printing. The struts would suffer from waviness or micro-cracks if the ratio is not at its optimum composition. Moreover, the metal powder should be handled within a closed environment, preferably under a glove box or vacuum hood, to protect the surfaces of metal particles from getting oxidized. Careful attention is required to optimize the flow behaviour of the dough so that could overcome the yield stress of the internal wall of the nozzle.

Question 10: Can we make porous Ti without struts using this extrusion based 3DP, if so how?

[Response] Porous Ti without struts using extrusion based 3D printing may be obtained if fugitive (starch or sugar in solid form) is included and strut to strut distance is zeroed down.

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