CHAPTER 5

COMPUTATIONAL FLUID DYNAMIC ANALYSIS AND BIOLOGICAL TESTING OF BONE SCAFFOLD

This chapter presents Computational Fluid Dynamic (CFD) analysis and in-vitro analysis of customised bone scaffold.

5.1 COMPUTATIONAL FLUID DYNAMIC ANALYSIS OF CUSTOMISED SCAFFOLD

CFD is a branch of fluid mechanics that uses numerical methods and algorithms to solve and analyze problems that involve fluid flows. Computers are used to perform the calculations required to simulate the interaction of liquids and gases with surfaces defined by boundary conditions.

Figure 5.1 (a) CAD model

Figure 5.1 (b) CFD model
Moreover, the numerical analysis offers a valuable alternative for the virtual testing of scaffold systems, which can give the visual output of a scaffold design working under different operating conditions. Numerical simulations serve as a useful tool for surgeons to optimize implant stability while treating bone fractures, and also save the surgical fixation time. (Devika & Arumaikkannu 2011).

The commercial CFD package, ANSYS CFX 11, was used to create contours of the Wall Shear Stress (WSS) magnitude through the scaffold. The fluid flows in from the top of the domain and comes out from the bottom and the other two lateral sides. Figure 5.1 (a) shows the CAD model of the scaffold structure, which was used for conducting the CFD analysis. Figure 5.1(b) shows the CFD mesh in which only the fluid domain is considered. The direction of the flow of the fluid has also been indicated. The inlet velocities applied to the scaffolds were varied between 0.2 mm/sec and 1 mm/sec. The fluid was modeled as a homogeneous Newtonian fluid. The fluid density of $1000 \text{ kg/m}^3$ and viscosity of $1.45 \times 10^{-3} \text{ Pa s}$ were similar to the cell culture medium (Dulbecco’s Modified Eagle Medium) at 37°C. (Bacabac et al 2005). Zero static pressure condition was applied at the outlet boundaries. The wall of fluid domain was taken as a no-slip condition (Chen et al 2010). CFD analyses were performed for the fifteen customised bone scaffolds enumerated in Table 3.1.

Computational fluid dynamics was applied to simulate the microfluid dynamics through the interconnected pores. However, the same flow rate through different pore architectures of scaffolds can bring about the different wall shear stress on the cells, which could result in different outcomes of tissue generation (Boschetti 2006).

The scaffold architecture also determines the level of fluid shear stress to which the cells are exposed, as a result of perfusion flow. Since this
quantity cannot be measured experimentally, computational fluid dynamics has been used to evaluate the local shear stress at the pore-level for real scaffold microarchitectures, which were modeled both in 2D and 3D, using histology and µCT imaging respectively. (Raimondi et al 2002, Porter et al 2005, Singh et al 2005).

5.2 BIOLOGICAL INVESTIGATION

Polyamide is biocompatible. The applied heat onto polyamide during the Additive Manufacturing process may produce cytotoxicity on the specimen. In addition to the cytotoxicity level, the studies of attachment of the cells on the polyamide components are of interest.

The MTT assay test can be used to verify the feasibility of selective laser sintered polyamide as scaffold material to fabricate the scaffold.

5.2.1 Cell line and culture

The osteoblast cell line (MG-63) was obtained from the National Centre for Cell Science (NCCS), Pune. The cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10 % FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 50 μg/ml CO₂ at 37 °C. (Serra et al 2013)

5.2.2 Reagents

Dulbecco’s Modified Eagle Medium (DMEM) was purchased from Hi Media Laboratories. Fetal Bovine Serum (FBS) was purchased from Cistron laboratories. Trypsin, Methyl Thiazolyl diphenyl- Tetrazolium bromide (MTT), and Dimethyl Sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals, Mumbai). All the other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.
5.2.3 **in-vitro testing**

The 0.8 mm diameter pore size scaffold was used to conduct *in-vitro* test as shown in Figure 5.2.

1) The scaffold prior to culturing with cells was autoclaved.

2) The scaffold was washed with 70 % ethanol twice (Saito et al (2012)), and then rinsed with PBS (phosphate buffered saline).

3) Then the scaffold was transferred carefully with forceps onto a sterile culture place (60 mm) and was conditioned in Dulbecco’s Modified Eagle Medium (DMEM) for two hours in 37 ºC incubator (with 5 % co$_2$ and 95 % humidity).(Chen et al 2006)

A CO$_2$ incubator operates on fairly simple parameters based on three elements: carbon dioxide (CO$_2$), temperature and relative humidity (RH). A scientist using a CO$_2$ incubator is trying to reproduce the mammalian environment (in-vivo)
outside of its natural state (in vitro). Therefore, the incubator combines three elements that create an environment needed for cells to thrive by establishing a balanced and controlled pH at 7.2-7.4: stable temperature at 37 °C, high RH at 95%, and controlled CO₂ level at 5%.

4) The medium was then drained off the scaffold, and the concentrated cell suspension (1×10⁶ cells/ml) was loaded onto the scaffold, and incubated at 37 °C in the incubator for two hours.

5) About 3 ml medium and 1 % FBS (Fetal Bovine Serum) (Sudarmadji et al (2011)) was added to the scaffold, and it was incubated for 3-7 days at 37 °C incubators.

5.2.4 Cell viability test

1) After 3 days of incubation of cells on the scaffold, the scaffold with the cells was prepared for cell viability assay – MTT (Methyl Thiazolyl diphenyl- Tetrazolium bromide).

(Principle of MTT- MTT is a yellow tetrazole that is reduced to purple formazan in living cells. A solubilization solution dimethyl sulfoxide (DMSO), an acidified ethanol solution is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a wavelength 595 nm by a spectrophotometer).

2) The medium from the scaffold was drained and about 3 ml of PBS-Trypsin (0.25 %) in 2:1 dilution was added to the scaffold and flushed vigorously onto the scaffold, to flush out
the attached cells. The flushed PBS-Trypsin was collected in fresh 15 ml Falcon tubes. This washing was repeated twice.

3) The collected PBS-Trypsin was centrifuged at 1500 rpm for 20 minutes, and the pellet formed was carefully resuspended in 200 µl of DMEM medium and loaded onto the wells in a 96 well plate.

4) Steps 2 and 3 were performed on all the scaffolds.

5) Cells grown in a 6 well plate were also trypsinized and plated in the same 96 well plate. This was used as the positive control (cells not grown on the scaffold). Then, about 10 µl of MTT was added into each well, and incubated for 4 hours at 37 °C in an incubator in the dark.

6) After 4 hours the MTT with some PBS was flushed from each well and centrifuged again at 1500 rpm for 10 minutes. The supernatant was removed and the pellet was added in all the wells. 150 µl DMSO was added in each well and incubated for 30 minutes in the incubator. Then the colour formed was analysed in an ELISA reader at 595 nm wavelength.

7) The readings were noted down and analysed.
The absorbance at 570 nm was measured with a UV- Spectrophotometer, using wells without samples containing cells as blanks. The effect of the samples on the proliferation of MG-63 was expressed as the % cell viability, using the formula given in Equation 5.1.

\[
\text{% cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100
\]  

(5.1)

5.3 SUMMARY

In this chapter, Computational Fluid Dynamic (CFD) analysis and \textit{in-vitro} analysis of customised bone scaffold has been presented.