Figure 6.53 reports the morphology of cells after 72 hours from the seed. The shape changes respect to the previous times. It becomes visible the body cell with an increase of the their roughness. The white precipitates were analyzed by EDS analyses (data not reported), they contain sodium and chlorine and come from the culture media. The cell culture media that is composed by a physiological solution, which induces the precipitation of these ions on the Palamed® surface. Probably this change of cell morphology can be related to the apoptosis normally induced to confluence phenomena for long time culture in close and static conditions.

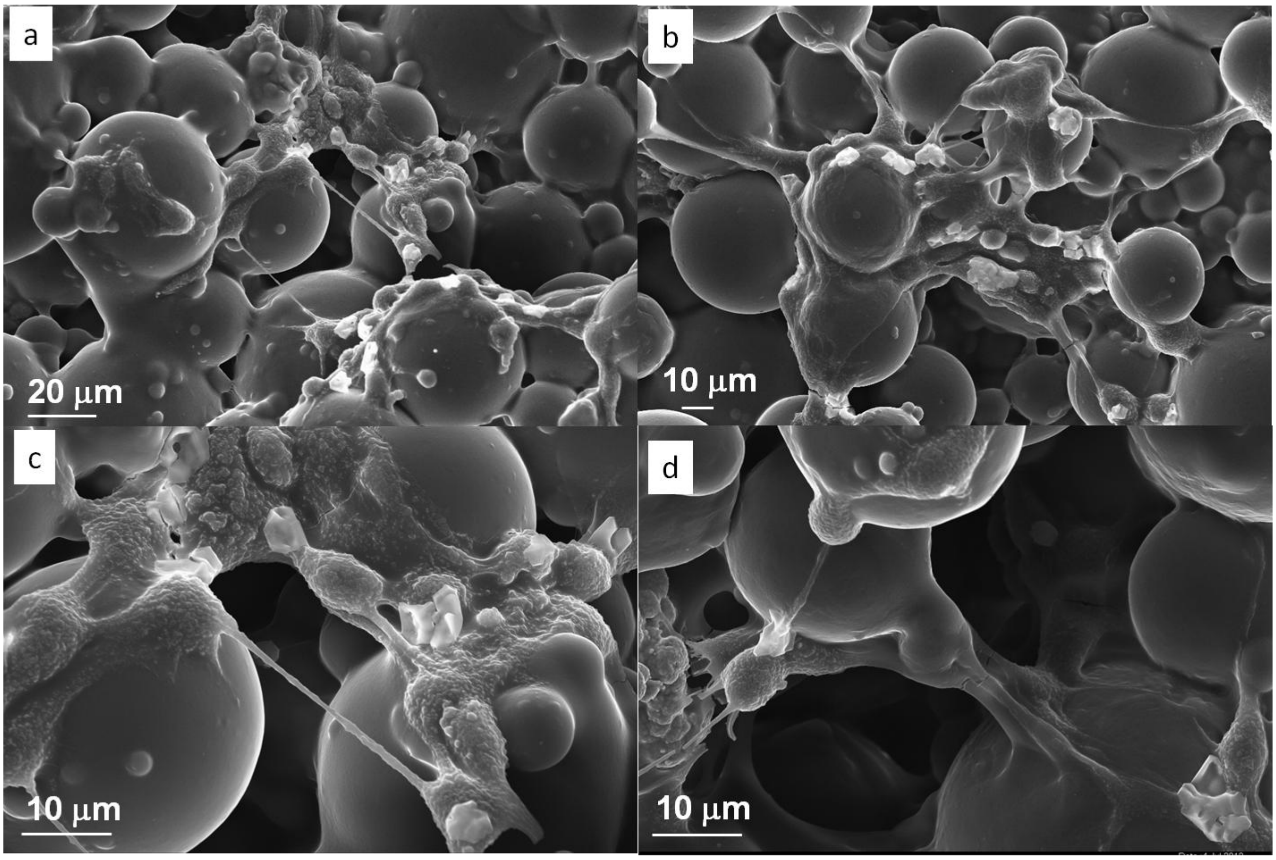


Figure 6.54: FESEM morphology of cells seeded on Palamed® after 72 hours of cultivation

A similar behavior is evaluated in figure 6.54 where MG63 appear with an evident cellular body with an increase of cell roughness.

The following results presented the morphology evaluation of cells on P10 sample at 24, 48 and 72 hours after cellular seed.

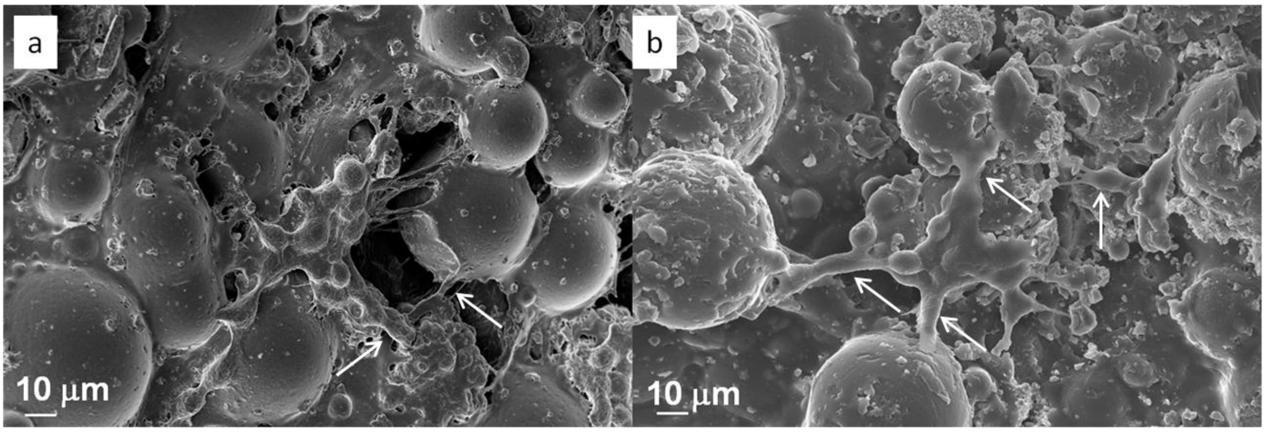


Figure 6.55: FESEM morphology of cells seeded on P10 after24 hours of culture

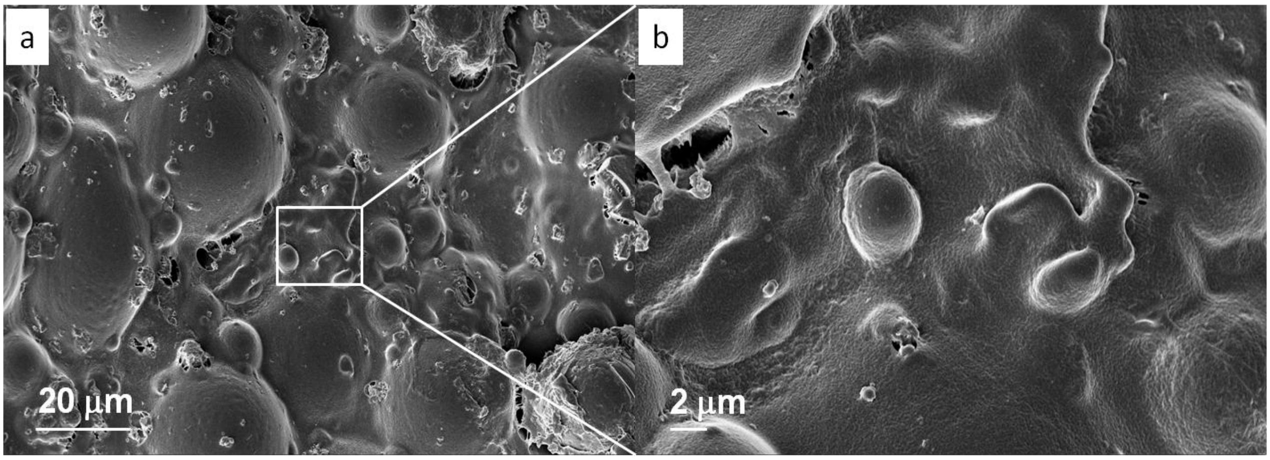


Figure 6.56: FESEM morphology of cells seeded on P10 after24 hours of cultivation

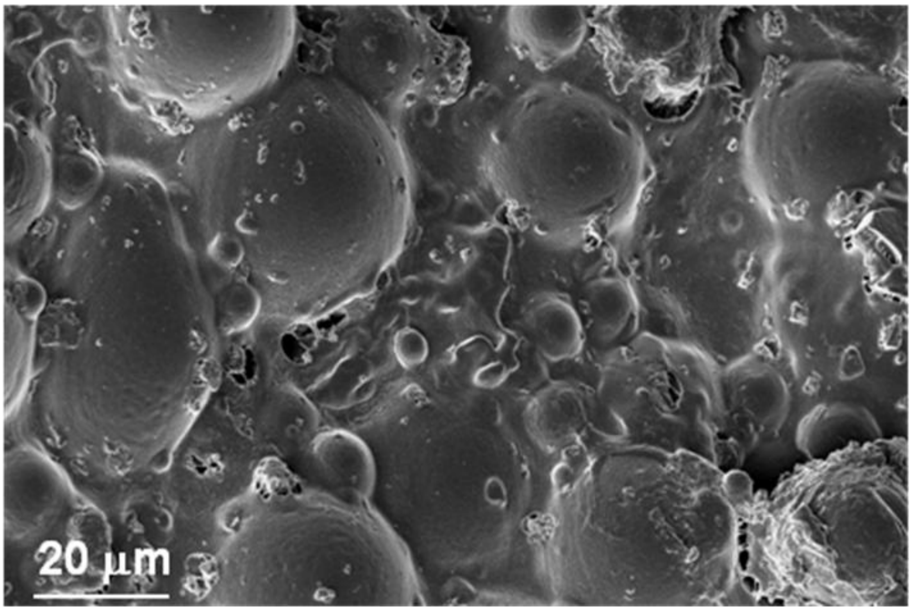


Figure 6.57: FESEM morphology of cells seeded on P10 after24 hours of cultivation

Figures 6.55 and 6.56 and 6.57 show a well spread of cell on the P10 surface, no cytotoxic effect is noticed. The cells proliferate in every point of material surface and show different morphologies .

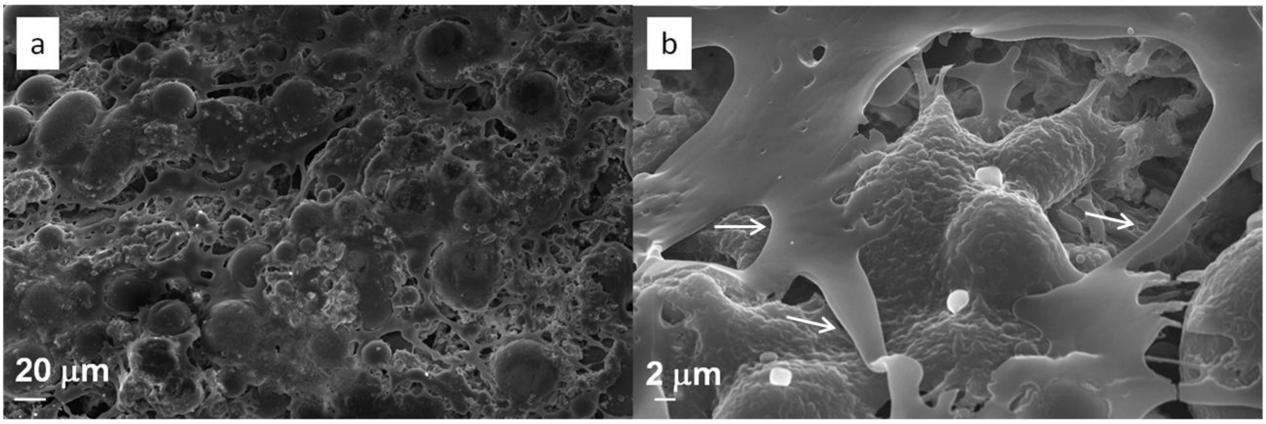


Figure 6.58: FESEM morphology of cells seeded on P10 after 48 hours of cultivation

Figure 6.58 reports a good cellular adhesion after 48 hour from seed on P10 sample. In the magnification in figure 6.58b it can be observed the develop of bridging connection between one cells and another. This is a very important result for the cells signaling and for the generation of a new tissue.

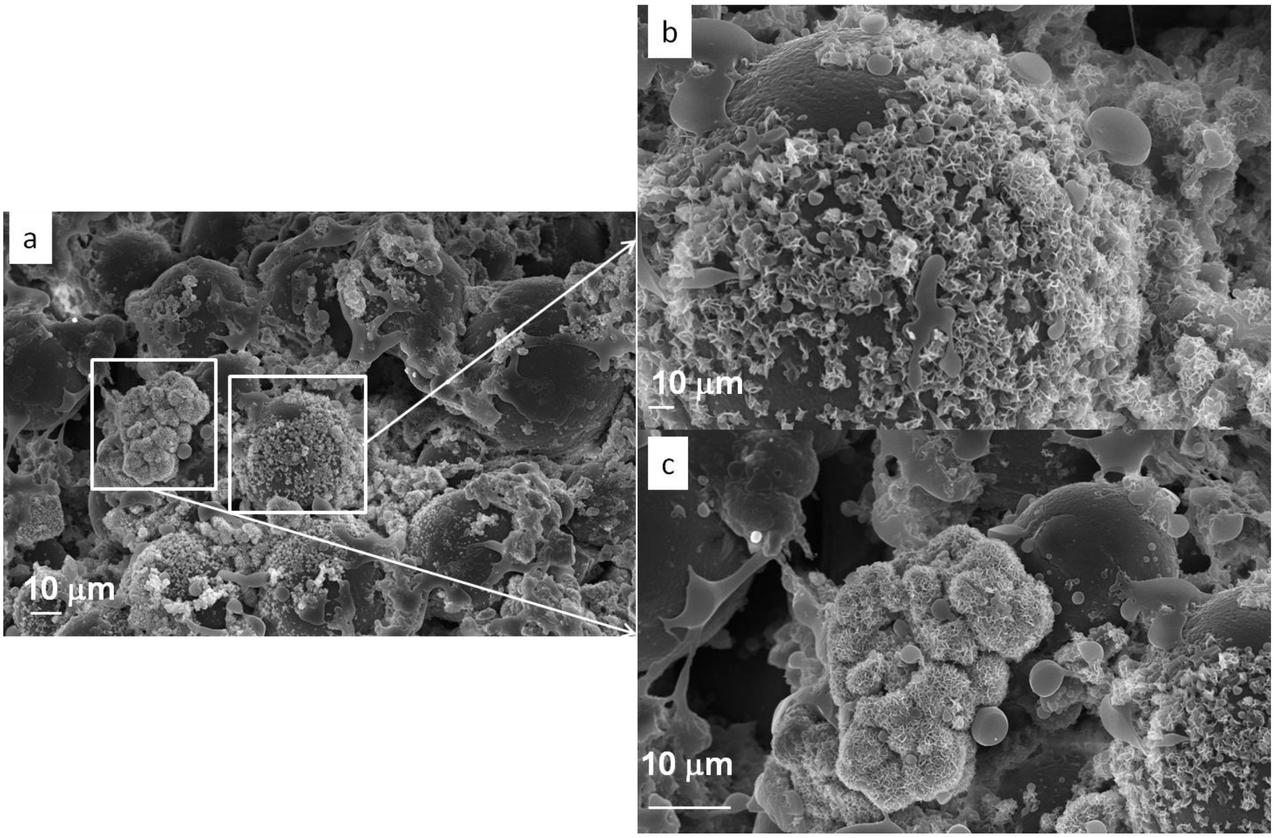


Figure 6.59: FESEM morphology of precipitates on P10 after 48 hours of cells cultivation

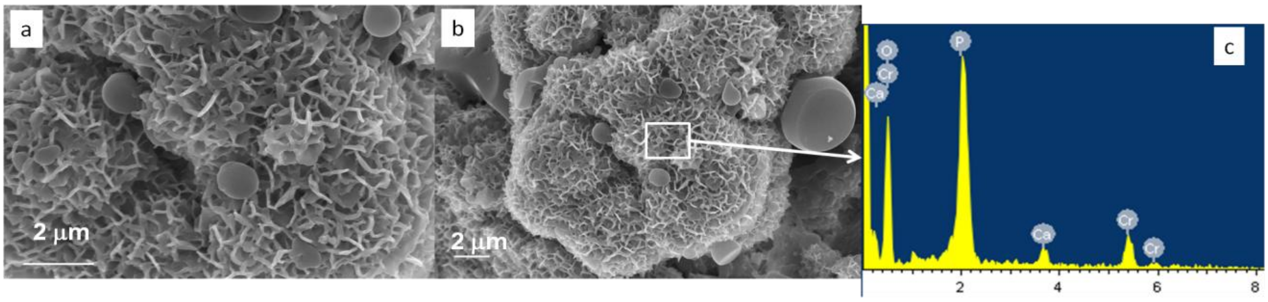


Figure 6.60: FESEM morphology of precipitates on P10 after 48 hours of cultivation

Figure 6.60 shows other images of precipitates with EDS analyses. In figure 6.60b it is reported a magnification of a precipitate, The EDS analysis performed on precipitates observed on P10 surface in different points documents the presence of calcium and phosphorus (figure 6.60c).

The formation of precipitates rich in Ca and P at early cell culture time-point is a very positive effect, since they have been not observed during the bioactivity test in SBF solution, so they presence suggest a synergic activity of the glass ceramic phase with the metabolic cycles of cells.

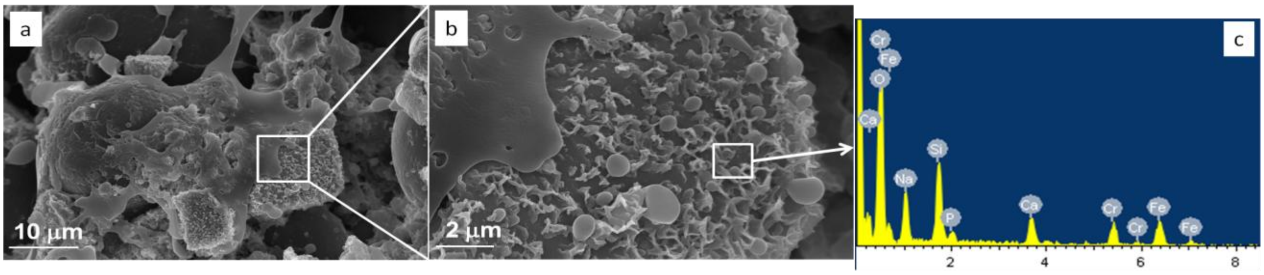


Figure 6.61: FESEM morphology of precipitates on P10 after 48 hours of cultivation

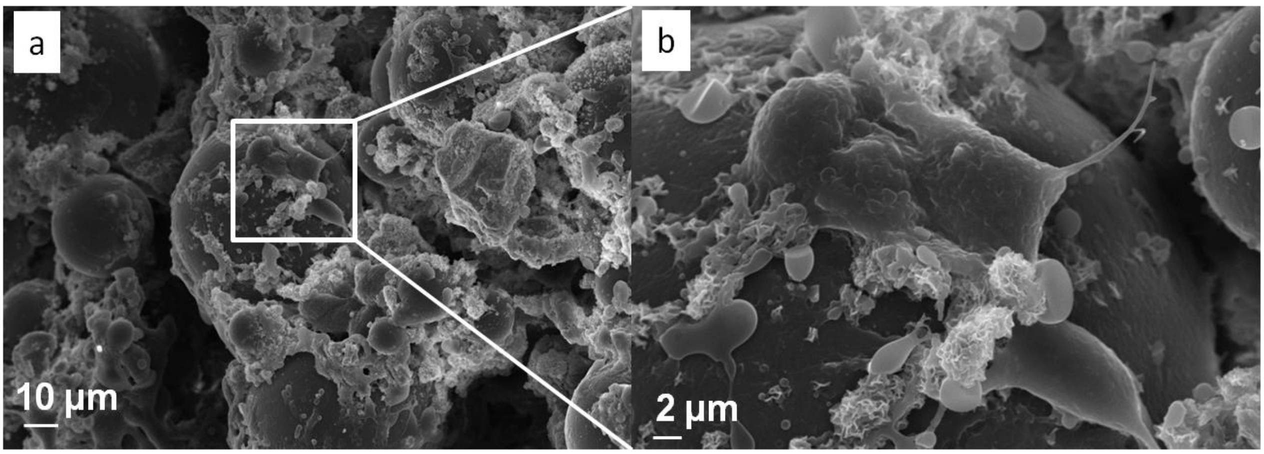


Figure 6.62: FESEM morphology of precipitates on P10 after 48 hours of cultivation

Figures 6.61a-b and 6.62a-b report similar results, where it is possible to observe a very good “osteo-induction” behavior, where the cells with the effect of glass ceramic have produced a lot of calcium and phosphorus nodules with a morphology very close to the hydroxyapatite one.

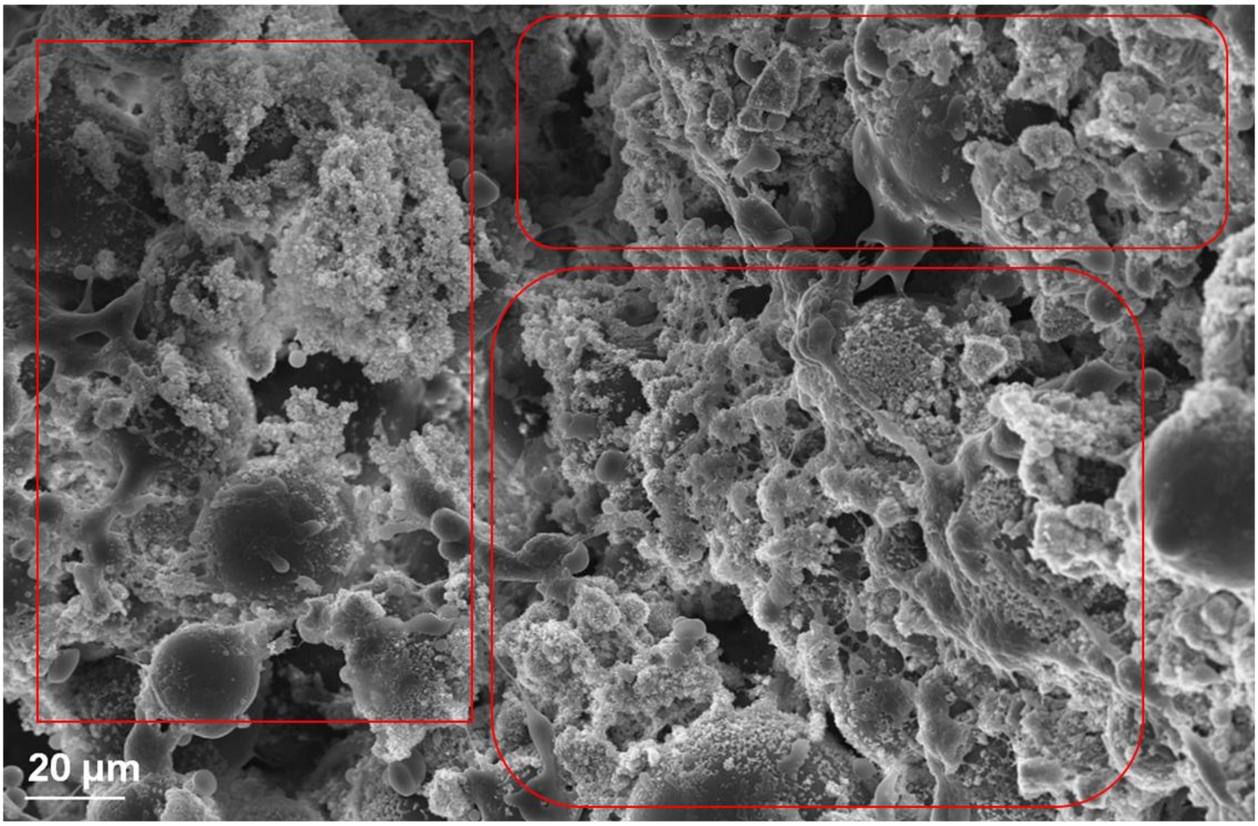


Figure 6.63: FESEM morphology of precipitates on P10 after 48 hours of cultivation

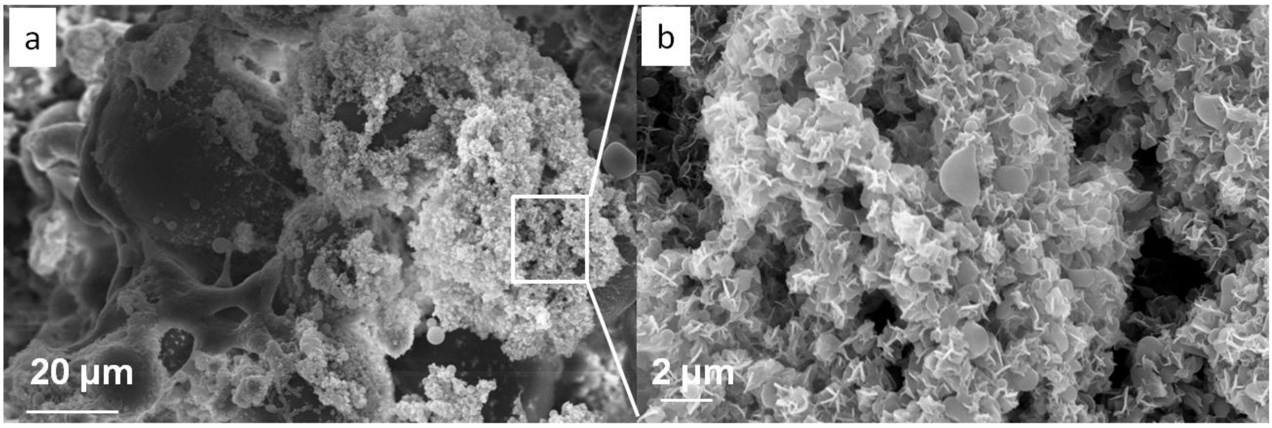


Figure 6. 64: Morphology observations of cells seeded on P10 cement after 48 hours of cultivation.

Figures 6.63 and 6.64 show the P10 surface completely covered with cells and precipitates (delimitated by red lines in fig. 6.63).

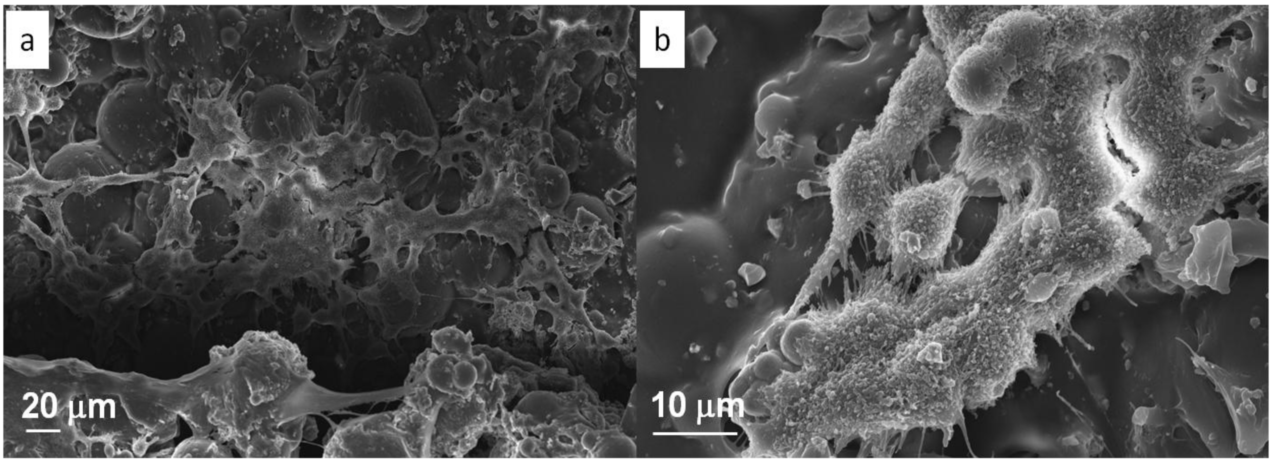


Figure 6. 65: Morphology observations of cells seeded on P10 cement after 72 hours of cultivation

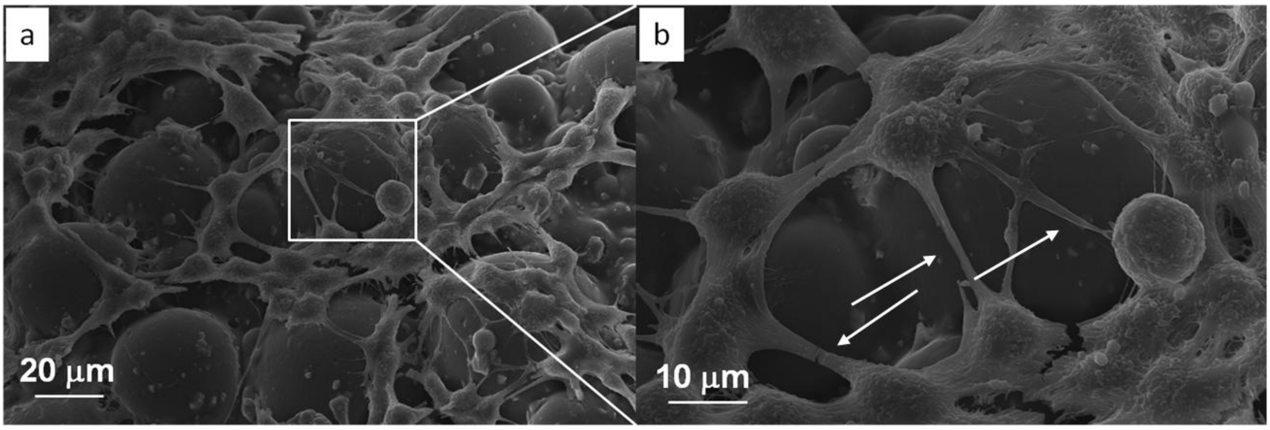


Figure 6. 66: Morphology observations of cells seeded on P10 cement after 72 hours of cultivation

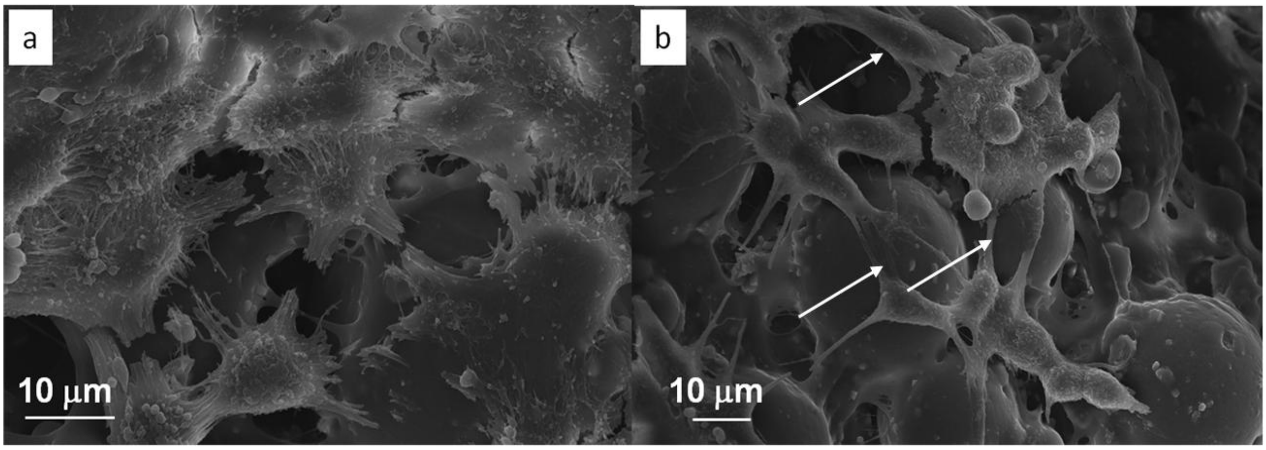


Figure 6. 67: Morphology observations of cells seeded on P10 cement after 72 hours of cultivation

At 72 hours, on P10 sample cell organization changes, in fact it observes a tridimensional structure (figures 6.65a-b-6.66a-b-6.67a-b), where several cells produce a highly interconnected tissue-like multilayer network with a first approach for the synthesis of a new tissue.

Data of the morphological characterization on P10 let assess that the glass-ceramic enrichment added to a commercial material (Palamed®) has not counteract cells adhesion and spread. Furthermore, cells show a very interesting ability to develop bridge-like complex structure (evidenced by arrows in figures 6.55a-b,6.58b,6.64b, 6.68b) between cells seeded onto close district; these structures are important pathways for cells signaling and represent a promising finding regarding the possibility to develop a cells-to-cells biochemical networking.

Figure 6.68 shows many different groups of cells that proliferate on the surface of P15 sample after 24 hours from the seeding.

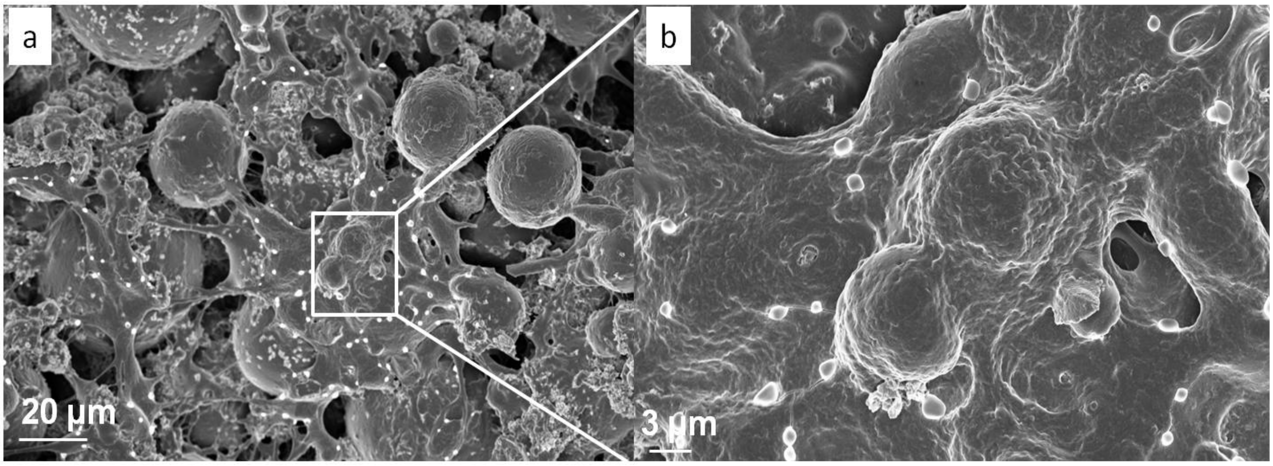


Figure 6.68: Morphology observations of cells seeded on P15 cement after 24 hours of cultivation

Figure 6.68 reports the morphology of MG63 on P15 after 24 h; it can be also noticed white dots, which are sodium chloride compound precipitated from the MEMS solution, in which the samples are contained.

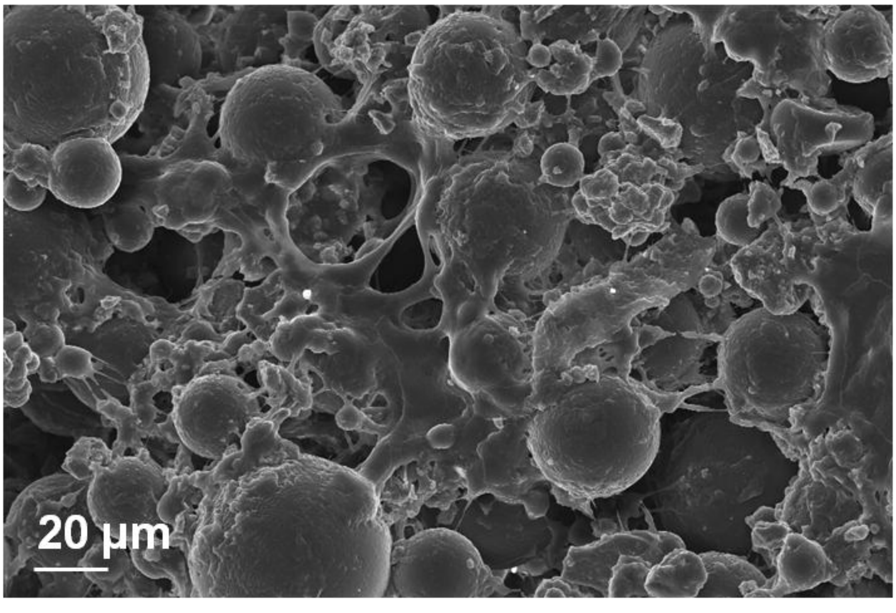


Figure 6. 69: Morphology observation of cells seeded on P15 cement after 24 hours of cultivation

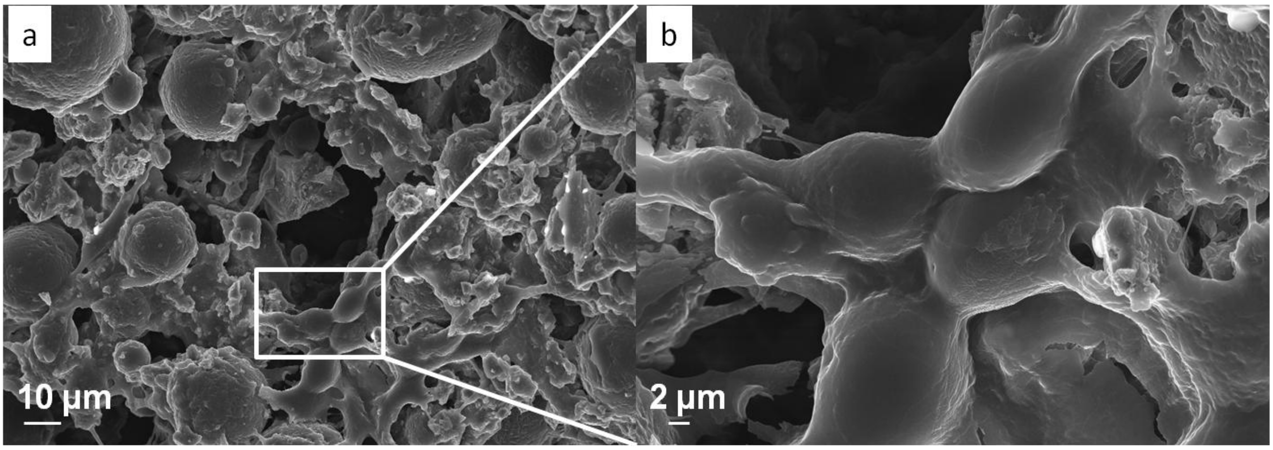


Figure 6.70: Morphology observations of cells seeded on P15 cement after 24 hours of cultivation

Figures 6.69 and 6.70a-b report an uniform distribution of cells on the P15 cement surface. The magnification in figure 6.68b evidences a different shape of cell's nucleus respect to the previous image 6.68b.

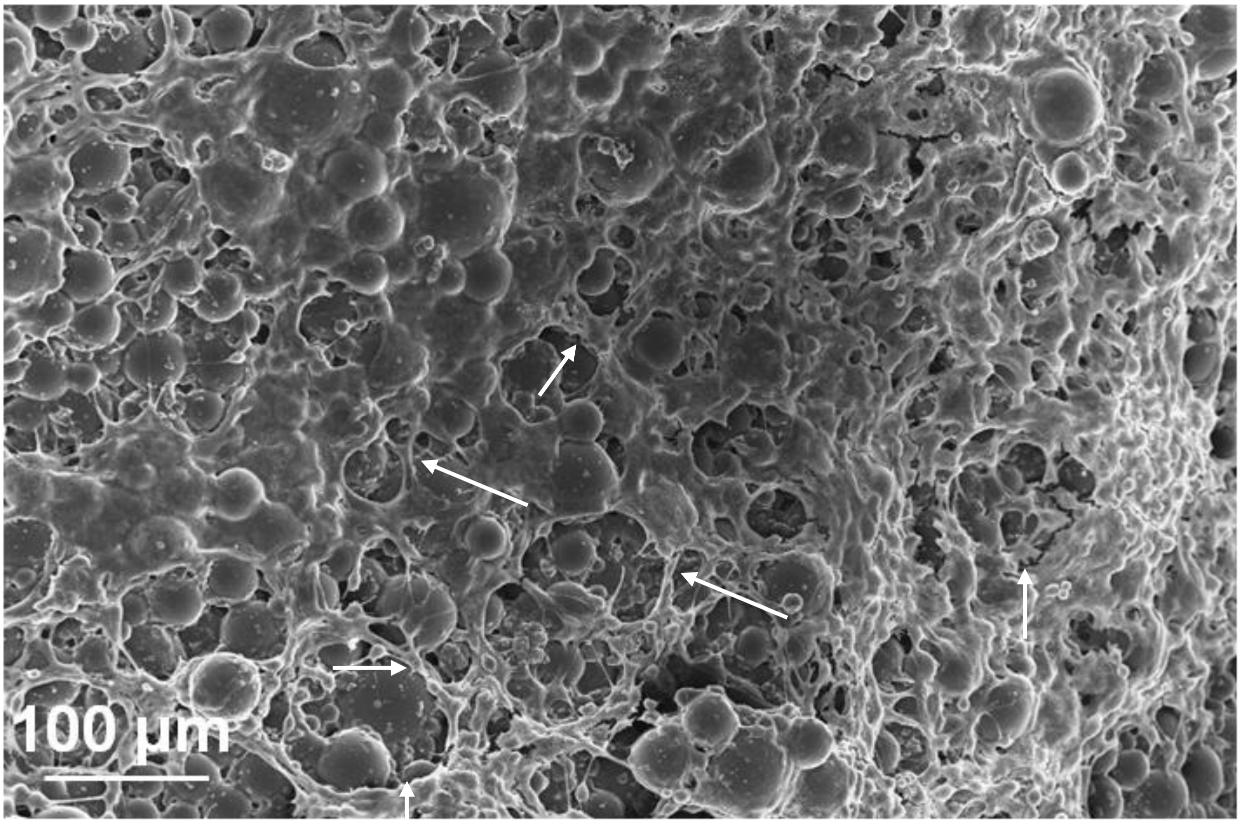


Figure 6. 71: Morphology observation of cells seeded on P15 cement after 48 hours of cultivation

Figure 6.71 reports an overview of P15 sample with a cover of cells on its surface. The cells are very well spread on the whole surface and they tend to form an uniform cover. A lot of bridge-like complex structure are seen as indicated by arrows.

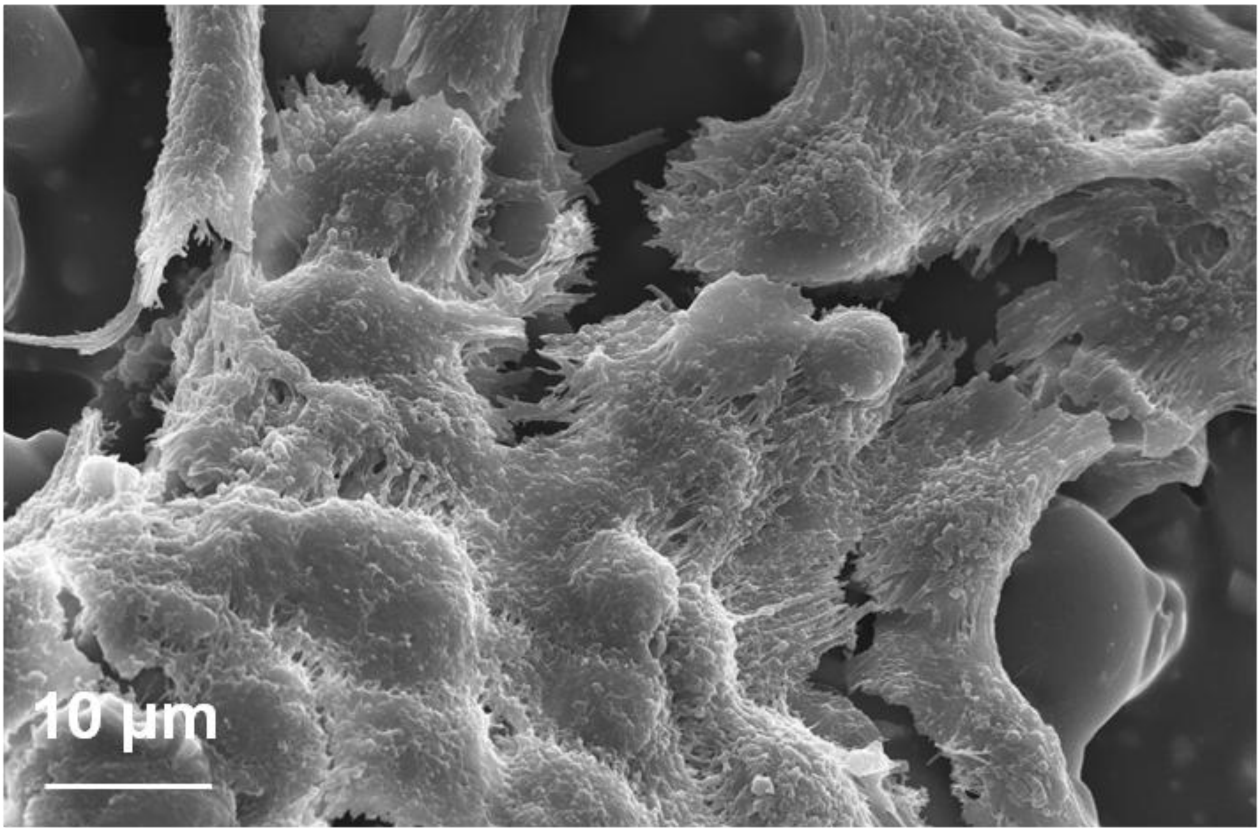


Figure 6. 72: Morphology observations of cells seeded on P15 cement after 48 hours of cultivation

Figure 6.72 documents a change in the cellular morphology after 48 h of culture. At this time-point the cells are not clear distinguishable and a first approach for the generation of a new tissue is observed. The cement surface is nearly not visible and this evidence the presence of a thick cell layer.

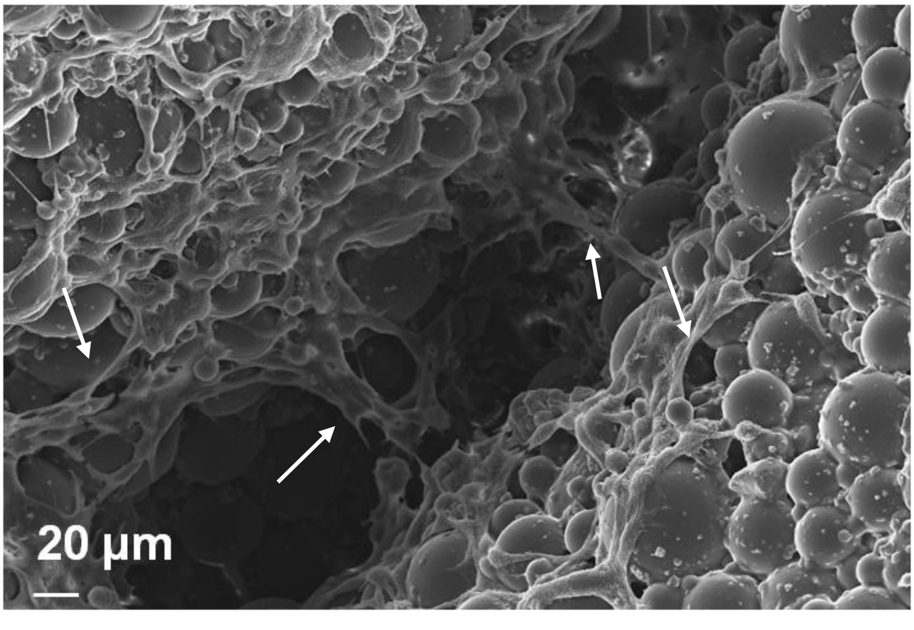


Figure 6. 73: Morphology observation of cells seeded on P15 cement after 48 hours of cultivation

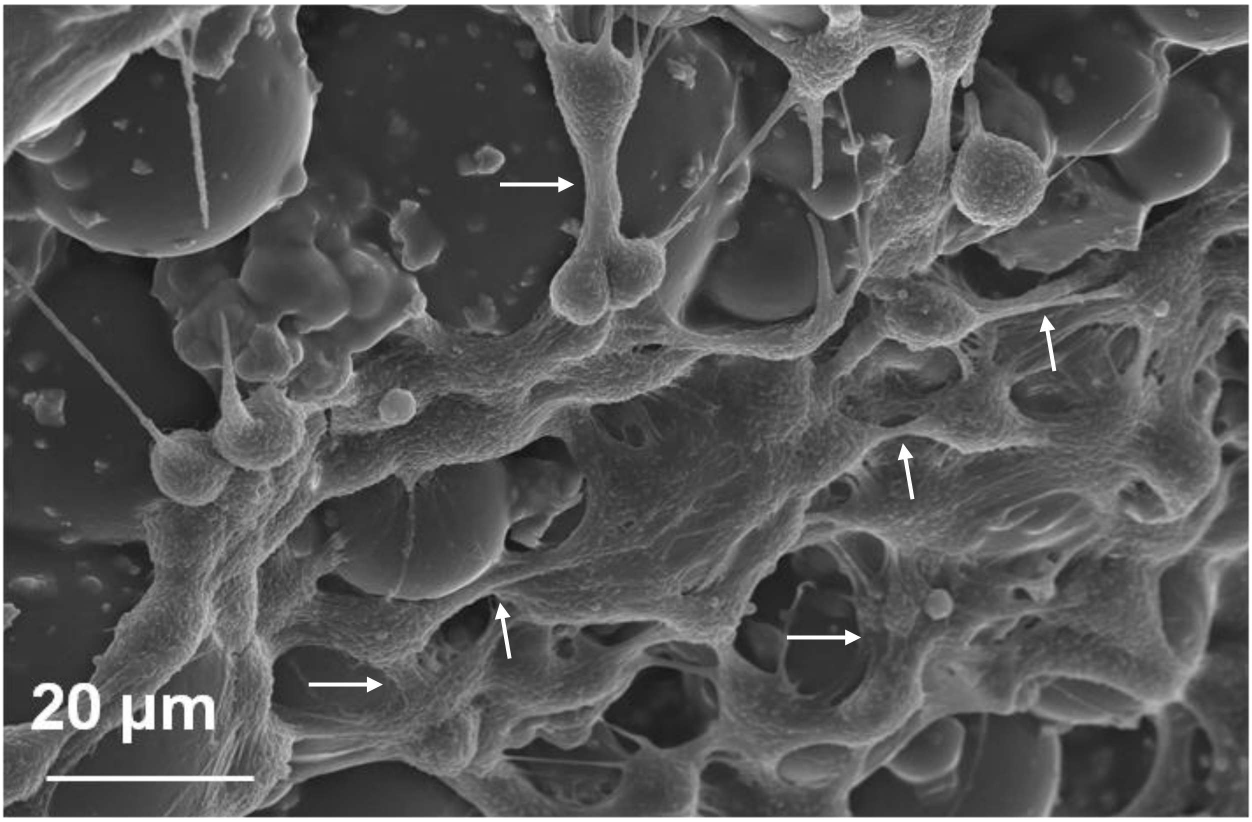


Figure 6. 74: Morphology observation of cells seeded on P15 cement after 48 hours of cultivation

Figures 6.73and 6.74 report a lot of bridging among cells, indicated with black arrows, and in the central part of the sample a 3D structure is observed. Similar behavior is noticed in figure 6.75a-b.

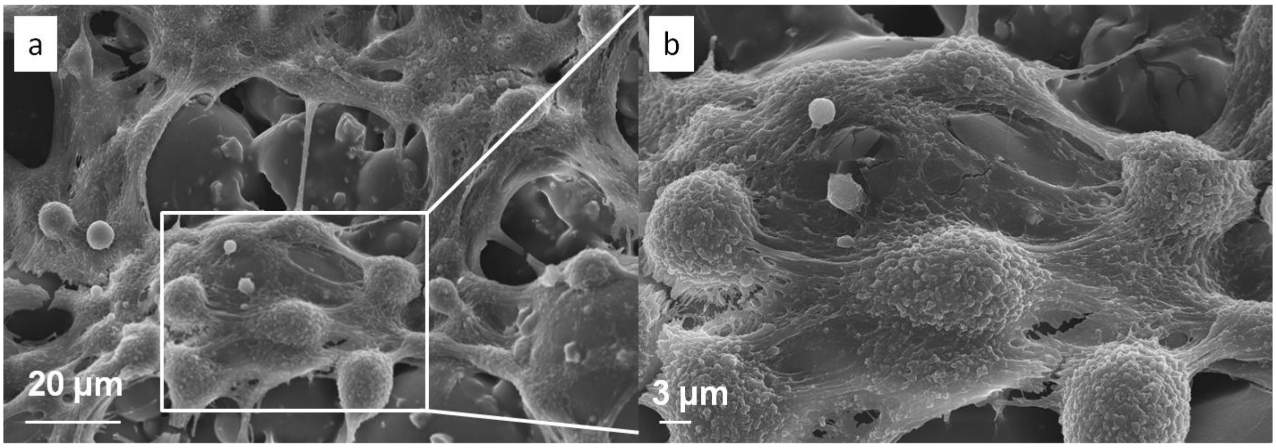


Figure 6.75: Morphology observations of cells seeded on P15 cement after 48 hours of cultivation

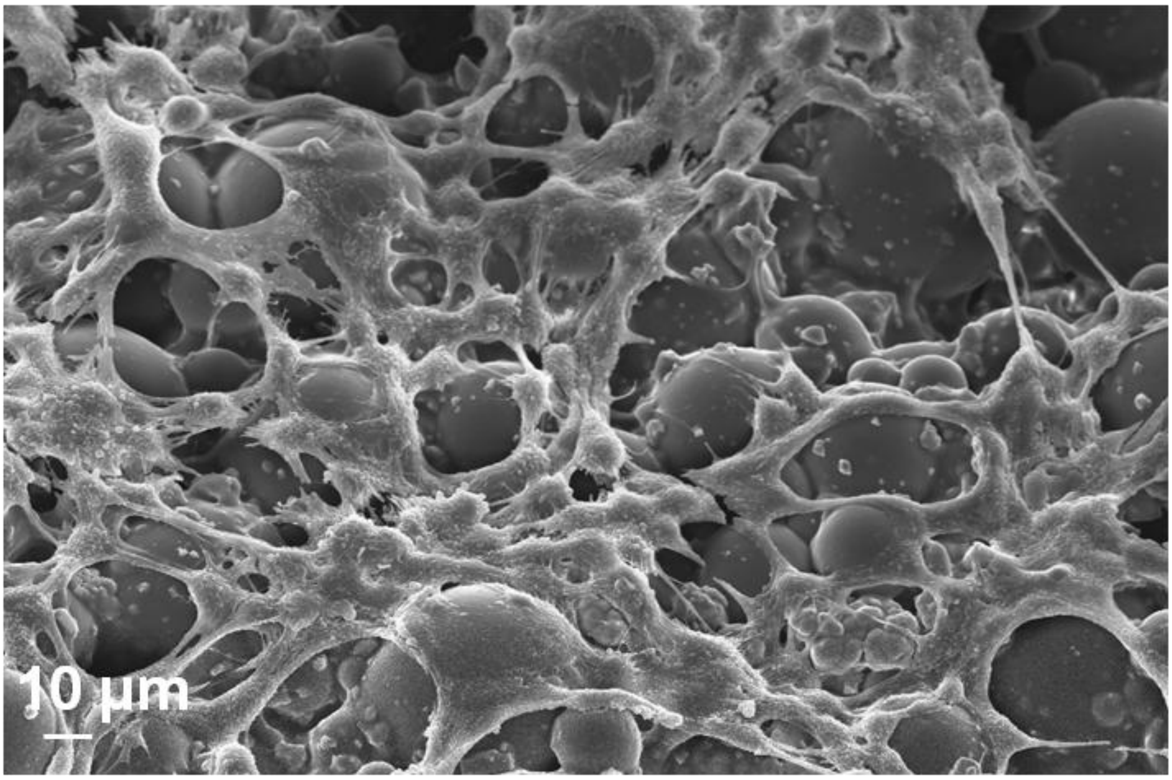


Figure 6. 76: Morphology observations of cells seeded on P15 cement after 48 hours of cultivation

After only 48 hours from the seeding on the composite, cells start to create a wide network connection with a lot of bridging like structure(figure 6.76). In agreement with the viability tests no cytotoxic effects are noticed.

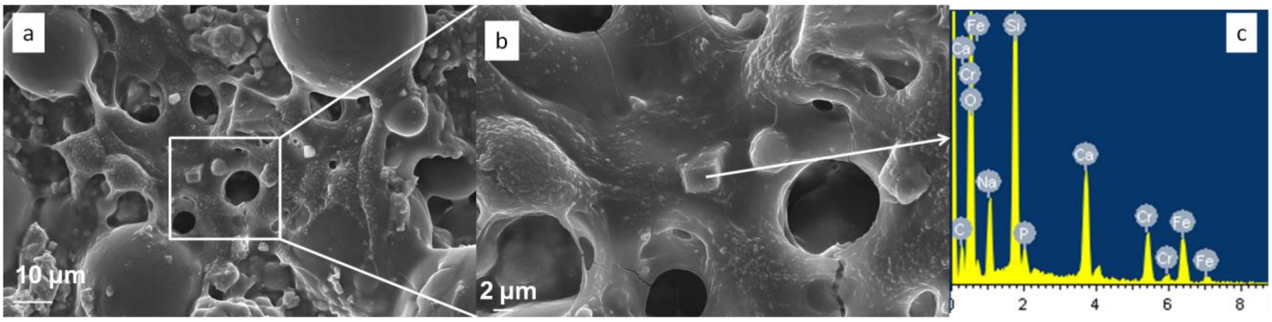


Figure 6. 77: Morphology observations of cells seeded on P15 cement after72 hours of cultivation

Figure 6.77 reports the cell's behavior after 72 hours from the seeding on P15 sample. It is not possible to distinguish one cell from another and in the magnification in figure 6.77b a tissue like structure appears. The EDS analysis reports the presence of a glass ceramic particle includes in the cellular matrix.

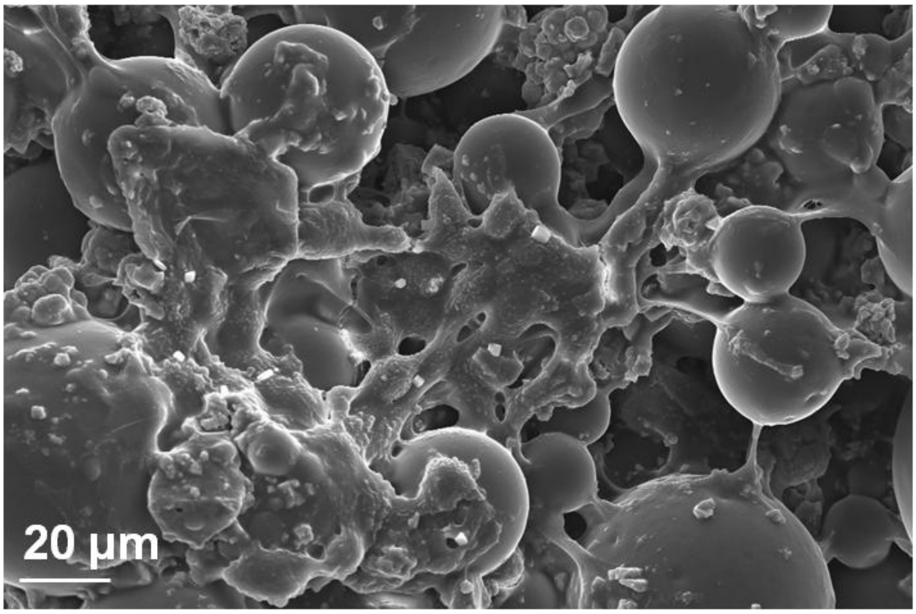


Figure 6. 78: Morphology observations of cells seeded on P15 cement after72 hours of cultivation

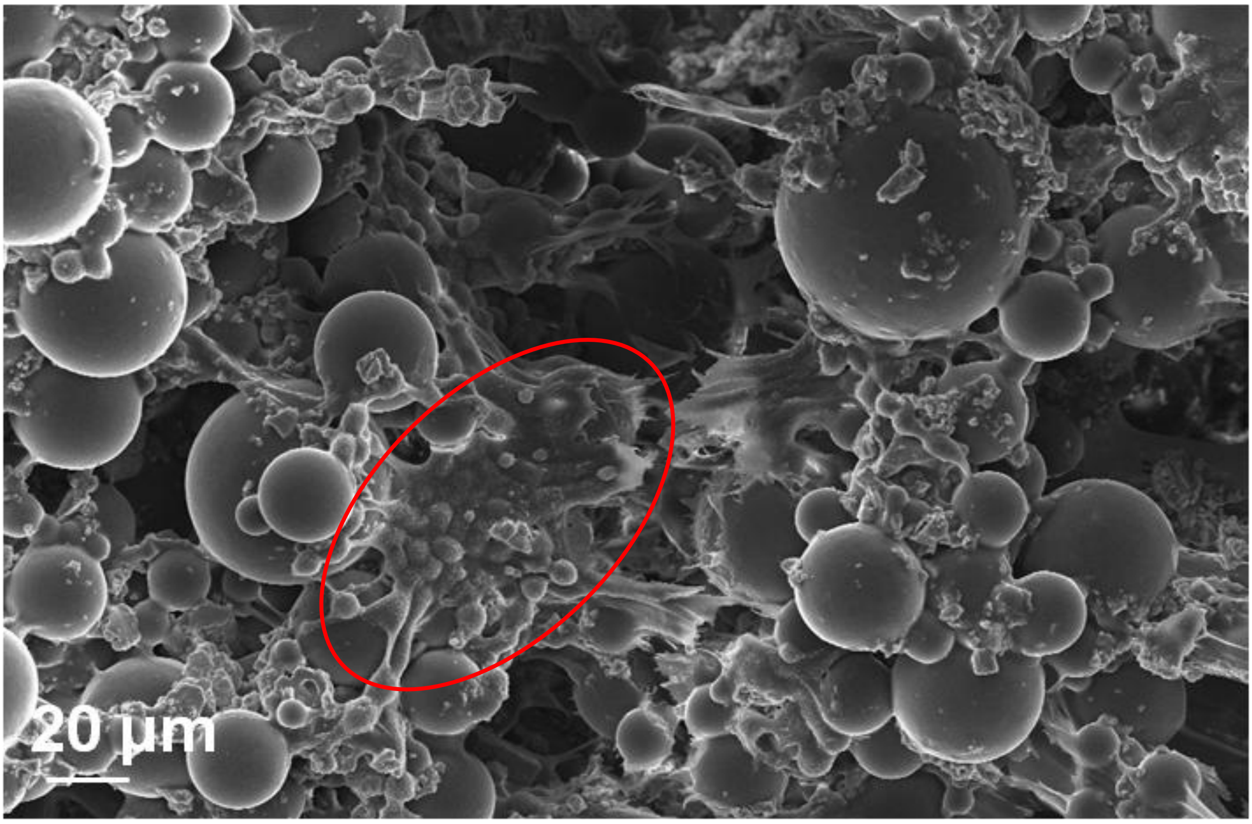


Figure 6. 79: Morphology observations of cells seeded on P15 cement after72 hours of cultivation

Similar situation appears in figures 6.78 and 6.79, where in the middle of the sample surface a wide 3D cell structure appears.

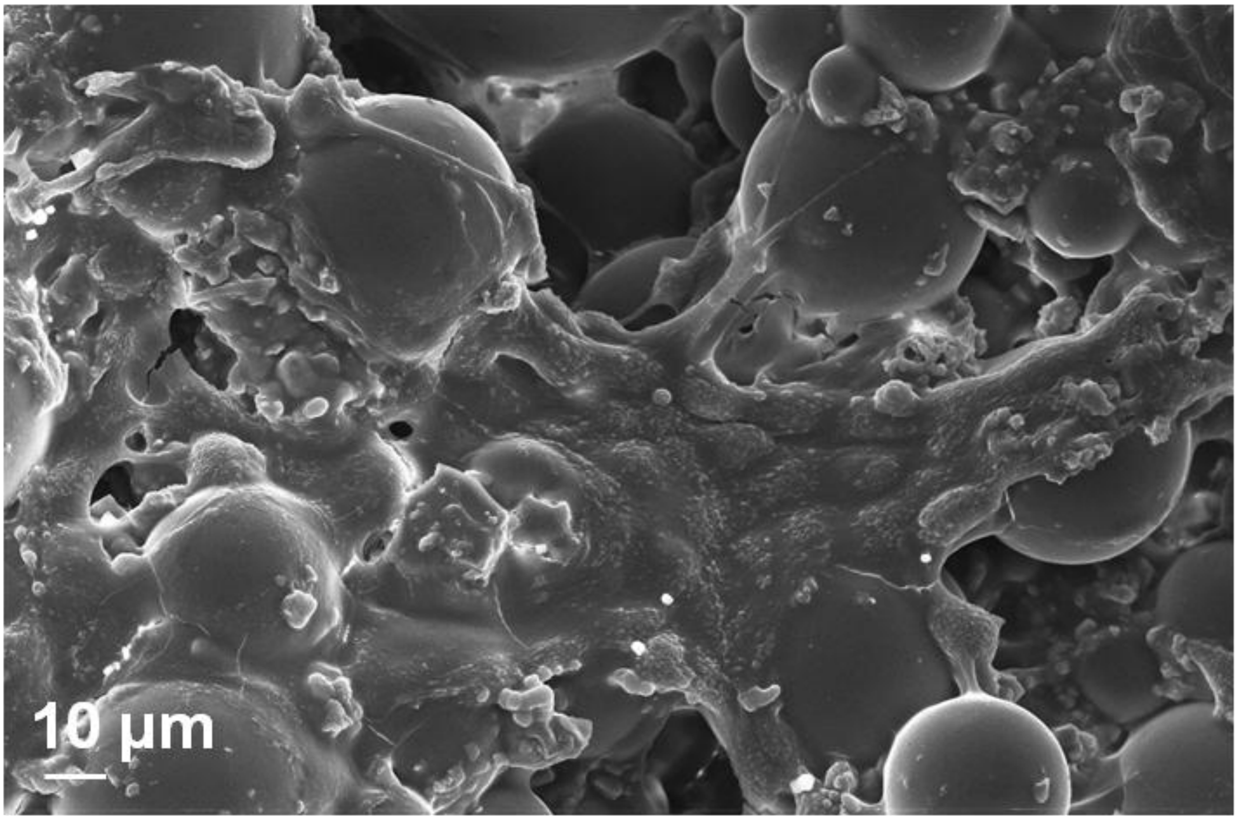


Figure 6. 80: Morphology observations of cells seeded on P15 cement after72 hours of cultivation

Even in figure 6.80 the shape of the cells changes and the union of many of them generate a 3D tissue like structure, which confirm the optimum behaviour of the material.

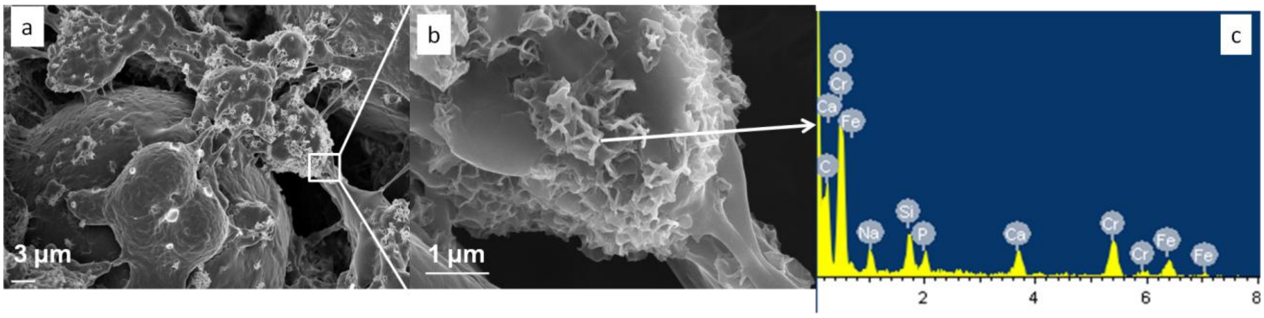


Figure 6. 81: Morphology observations of precipitates on P15 cement after24 hours of cultivation

Moreover even for P15 samples many calcium and phosphorus precipitates are noticed after 24 hours from the seeding of cells on the composite surface. The acicular shape of the needles can be supposed the presence of hydroxyapatite particle which are the results of a synergic effect of the glass ceramic and the cell metabolism (figure 6.81). The presence of a favorable osteosarcoma cells proliferation have induced the precipitation of a mineral bone part. A very import role was played by the glass ceramic which composition take inspiration by Bioglass for the high osteo-integration property.

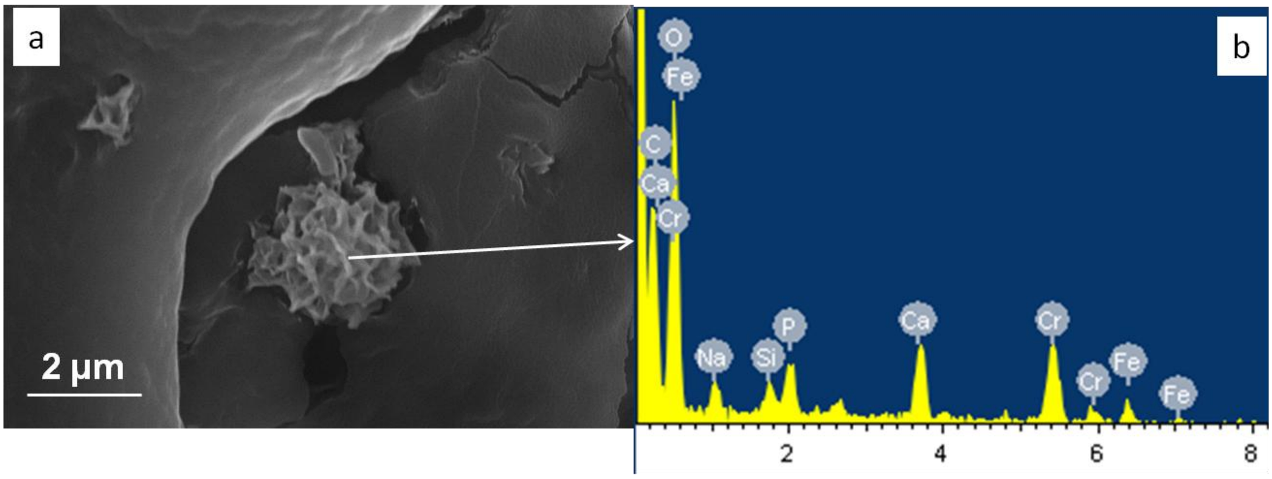


Figure 6. 82: Morphology observations of precipitates on P15 cement after24 hours of cultivation

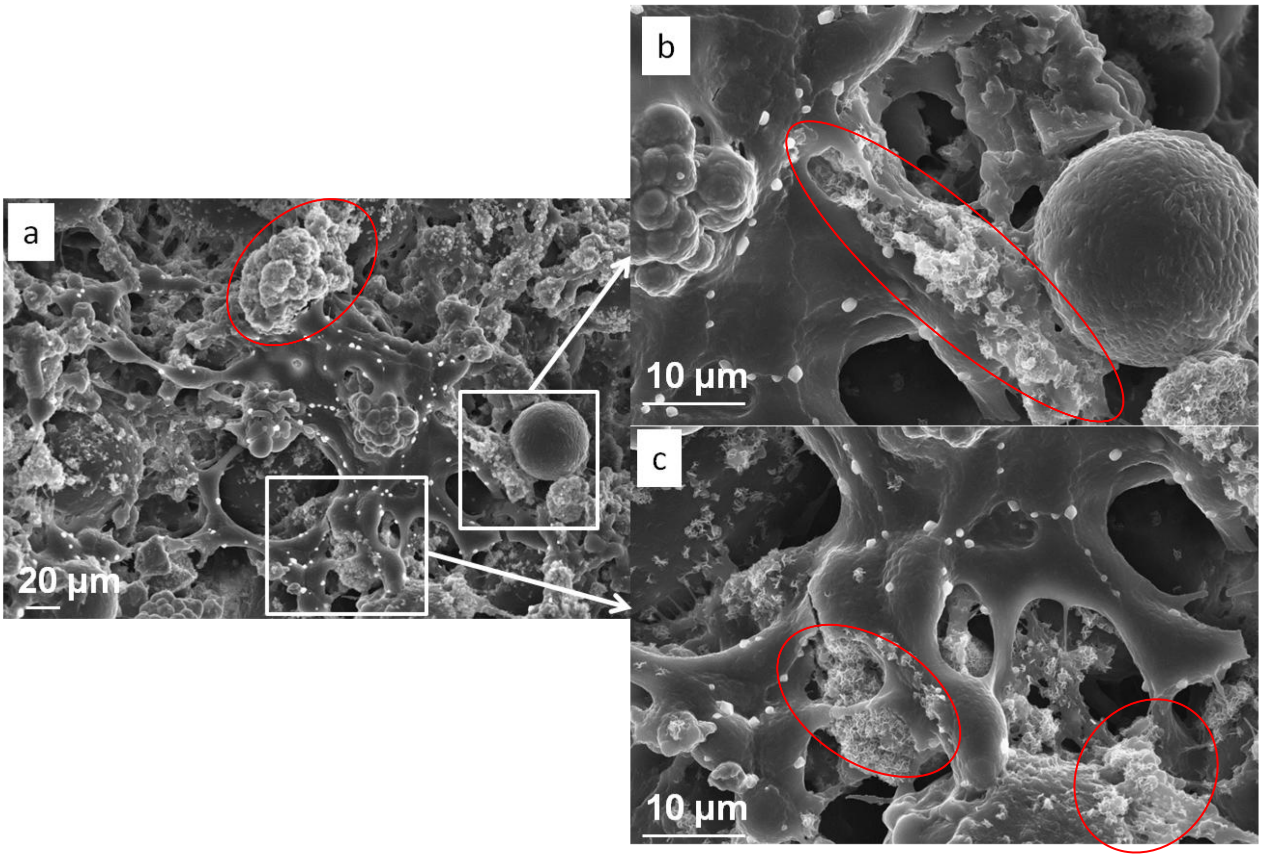


Figure 6. 83: Morphology observations of precipitates on P15 cement after24 hours of cultivation

As confirmed in figures 6.82 and 6.83 calcium and phosphorus precipitates are presented in different part of the sample surface: near cells and on composite surface, where probably there are exposed glass ceramic particles. The glass ceramic ion exchange with medium and metabolic activity of cells have certainly favored the HAp precursor precipitation.

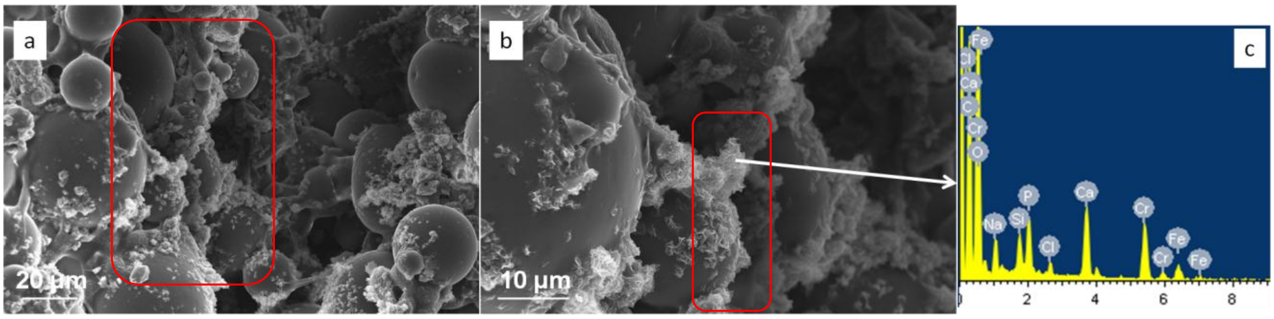


Figure 6.84: Morphology observations of precipitates on P15 cement after 72 hours of cultivation

After 72 hours from seeding of MG63 the HAp precursor were still seen as demonstrate even by the compositional analysis in figure 6.84c.

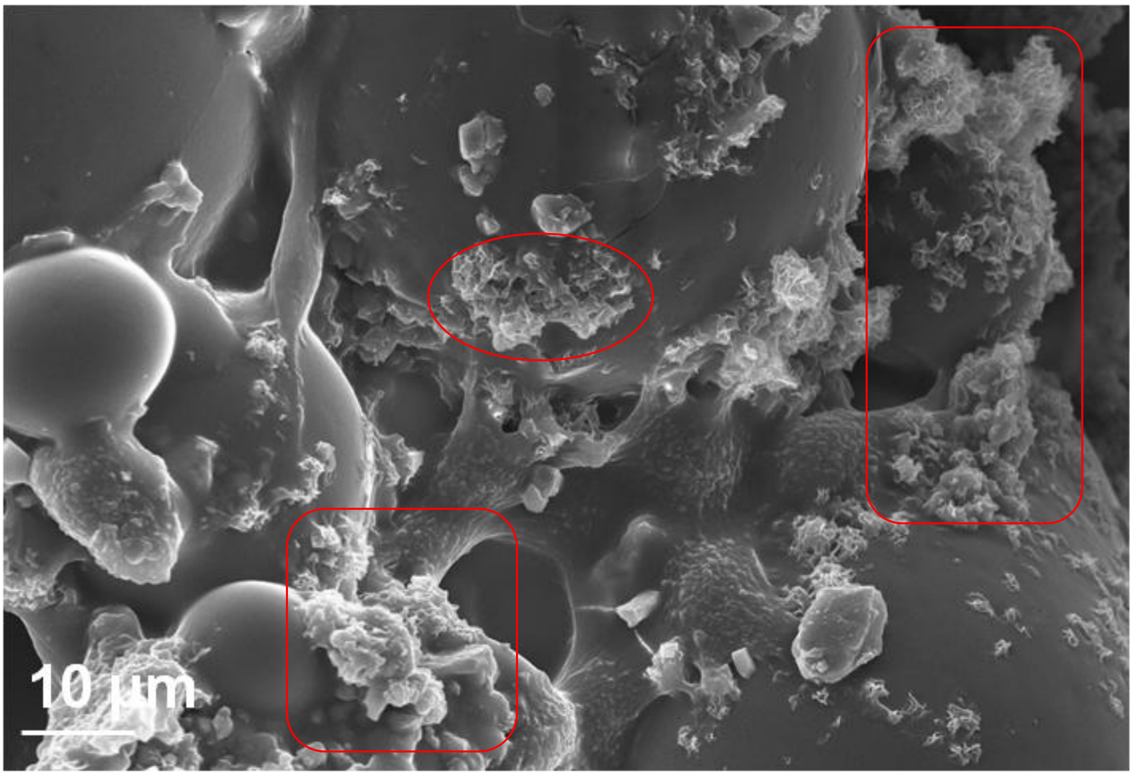


Figure 6. 85: Morphology observations of precipitates on P15 cement after 72 hours of cultivation

The combined effect of the glass ceramic and the cells was also observed in figure 6.85, where a perfect interaction between cells and material is demonstrated without toxic effects.

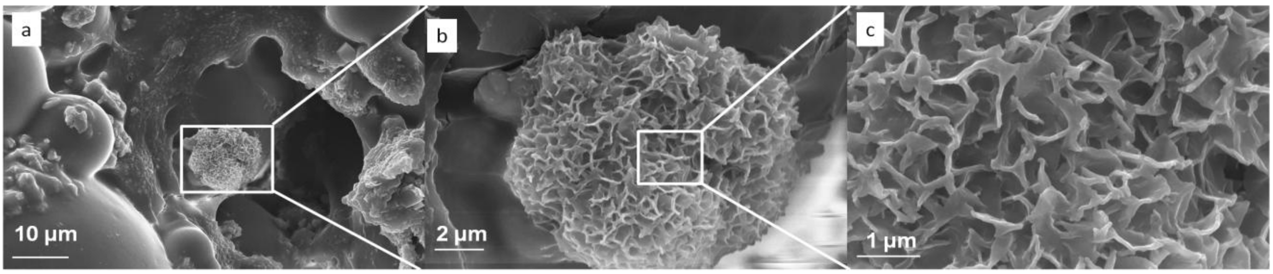


Figure 6. 86: Morphology observations of precipitates on P15 cement after 72 hours of cultivation

Figure 6.86a-b-c reported a sequence of magnification of a precipitate with a typical hydroxyapatite morphology and structure present in a porosity of P15 sample. The needle like shape is evident in figure 6.86c.

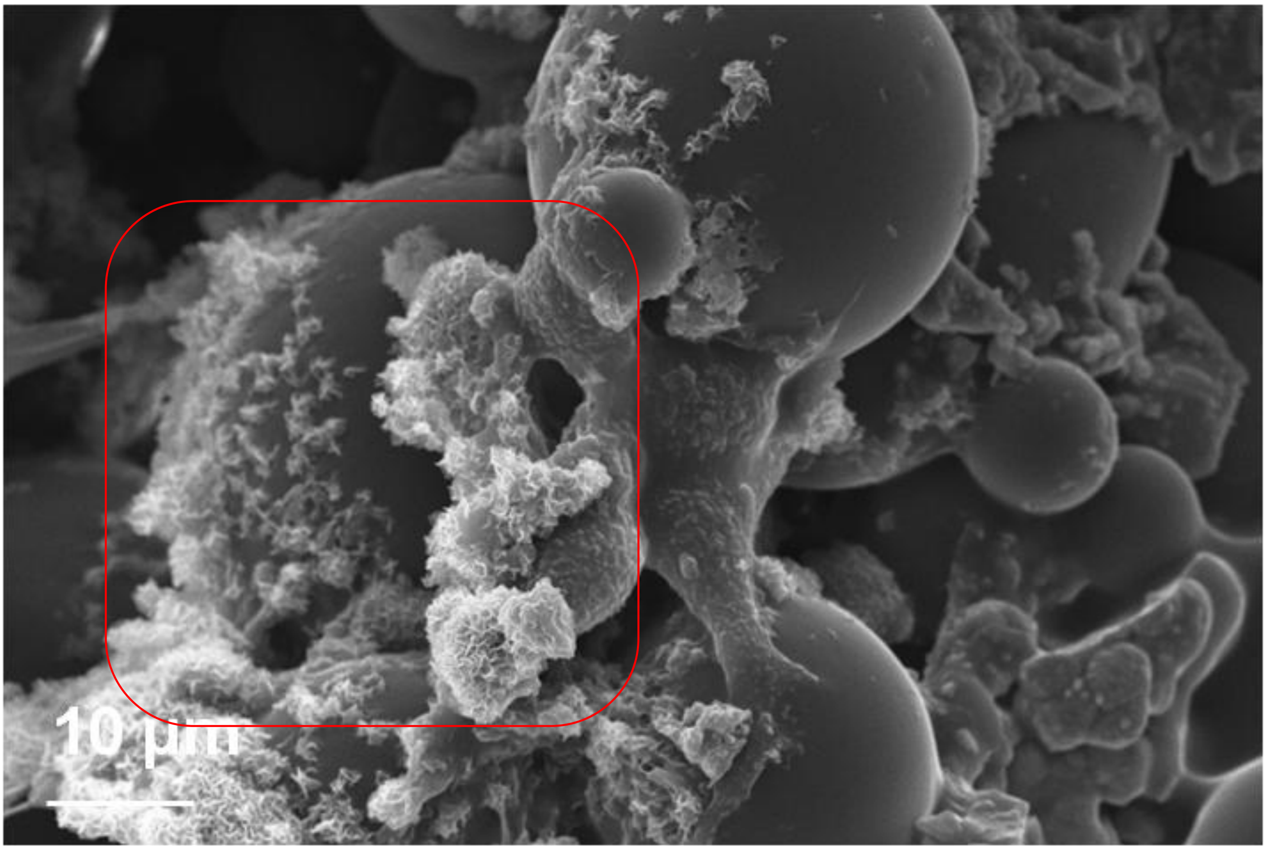


Figure 6. 87: Morphology observations of precipitates on P15 cement after 72 hours of cultivation

Figure 6.87 reports another image that demonstrates the biocompatible behavior of this sample. Again, the production of calcium-phosphorous precipitates confirms the synergistic effect between the bioactivity of the material and cell activity.

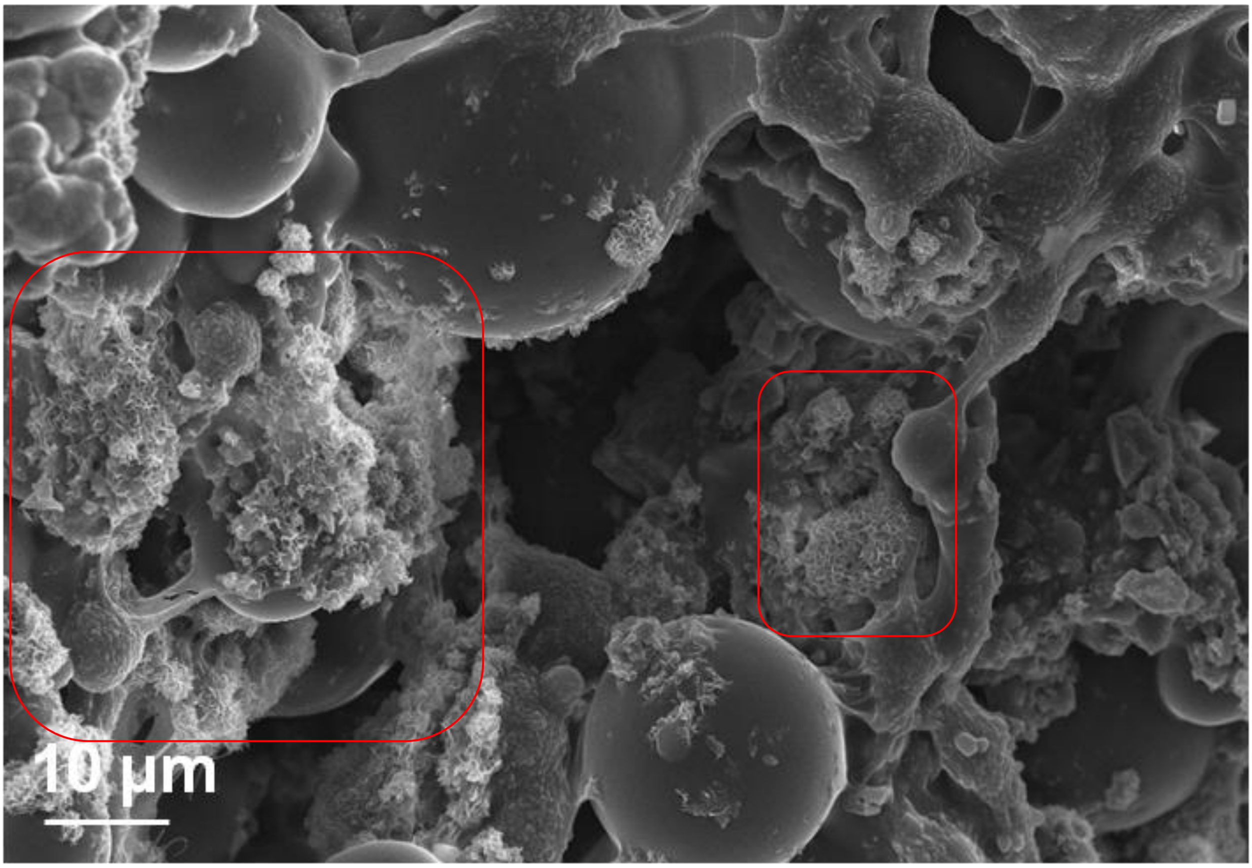


Figure 6. 88: Morphology observations of precipitates on P15 cement after 72 hours of cultivation

Similar behavior to the previous image is observed in figure 6.88. The red lines evidence the presence of precipitates and the cells in the same spaces.

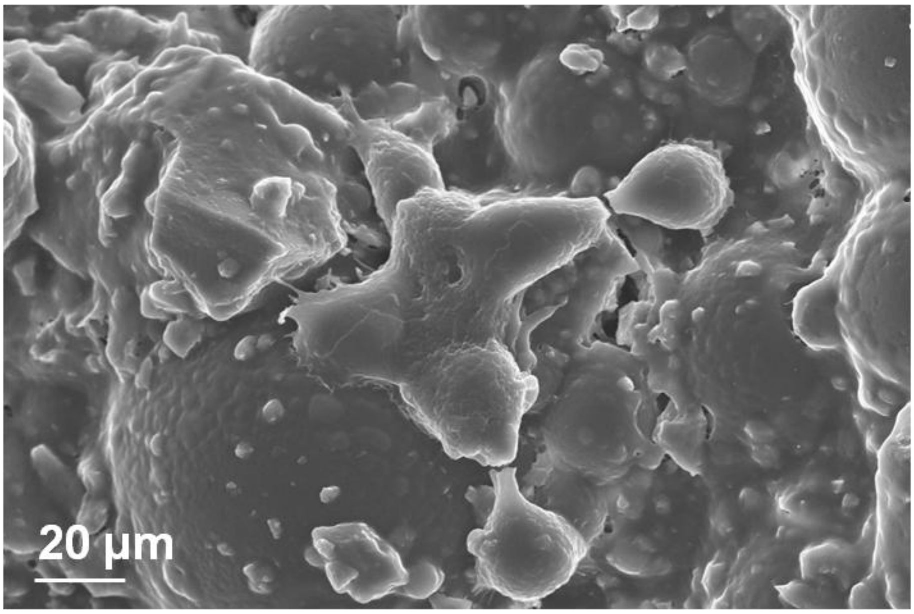


Figure 6.89: Morphology observations of cells seeded on P20 cement after 24 hours of cultivation

Figures 6.89 and 6.90 report morphology and cells proliferation on P20 sample. Even for this specimen no cytotoxic effect is noticed as confirmed by viability tests.

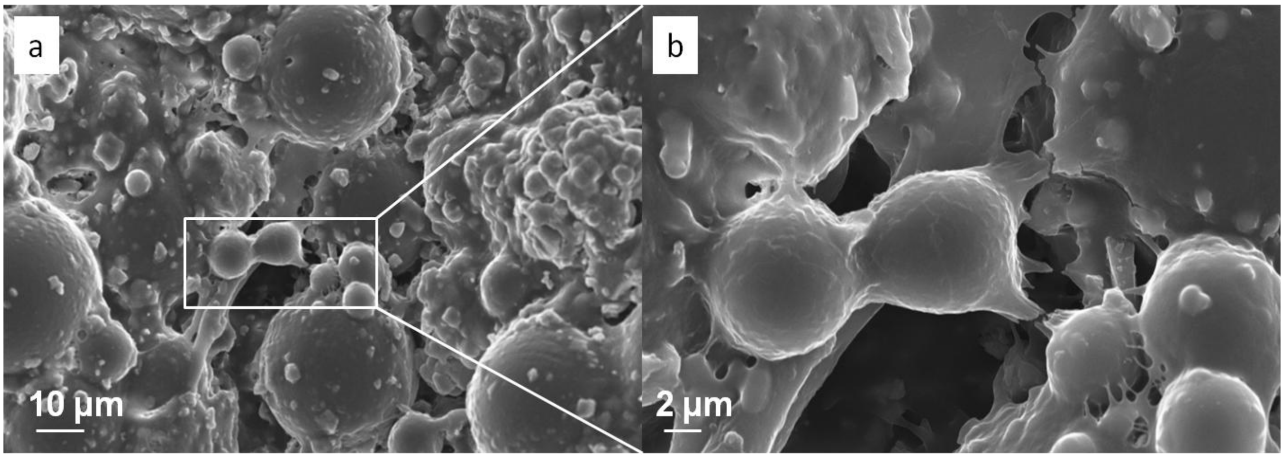


Figure 6. 90: Morphology observations of cells seeded on P20 cement after24 hours of cultivation

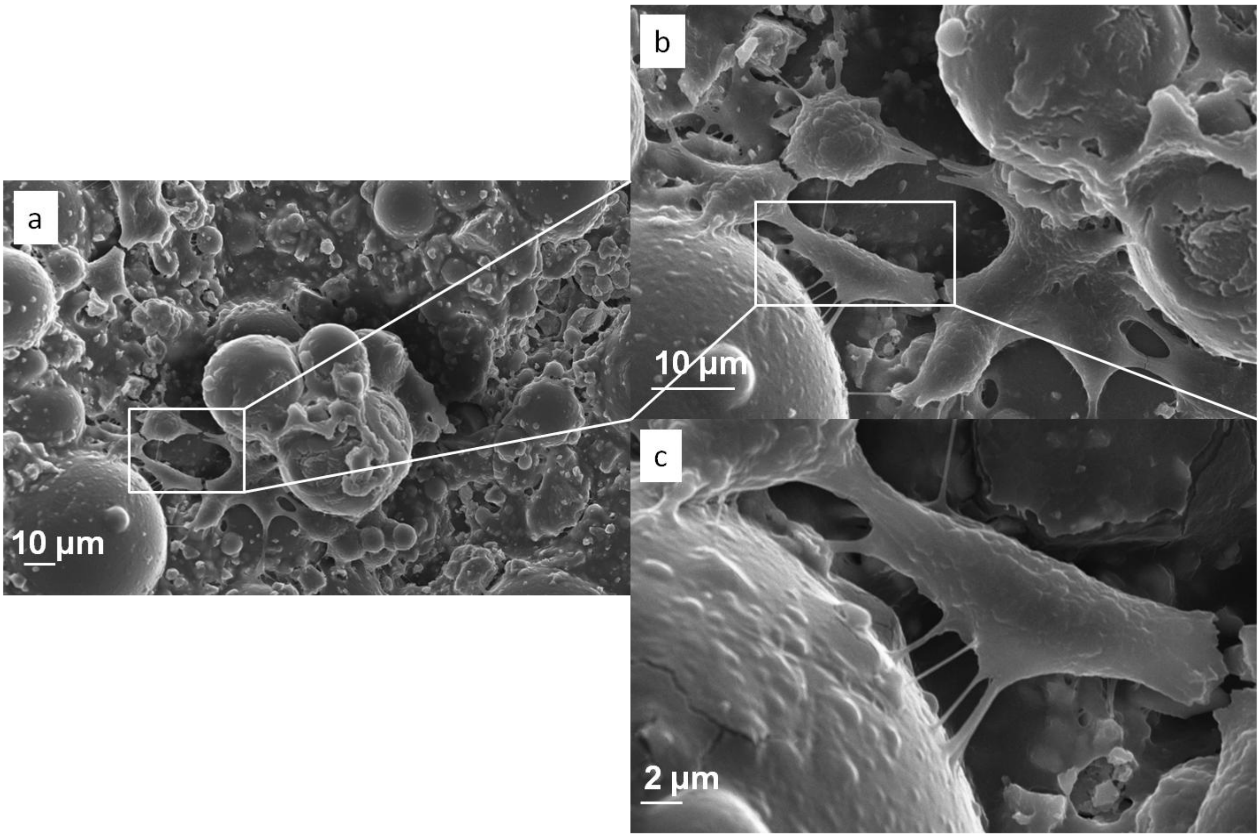
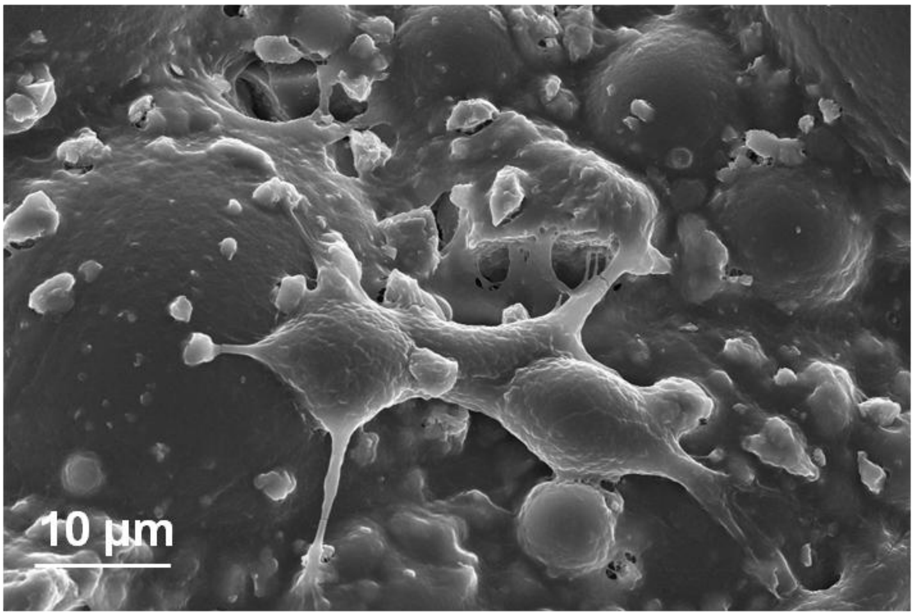
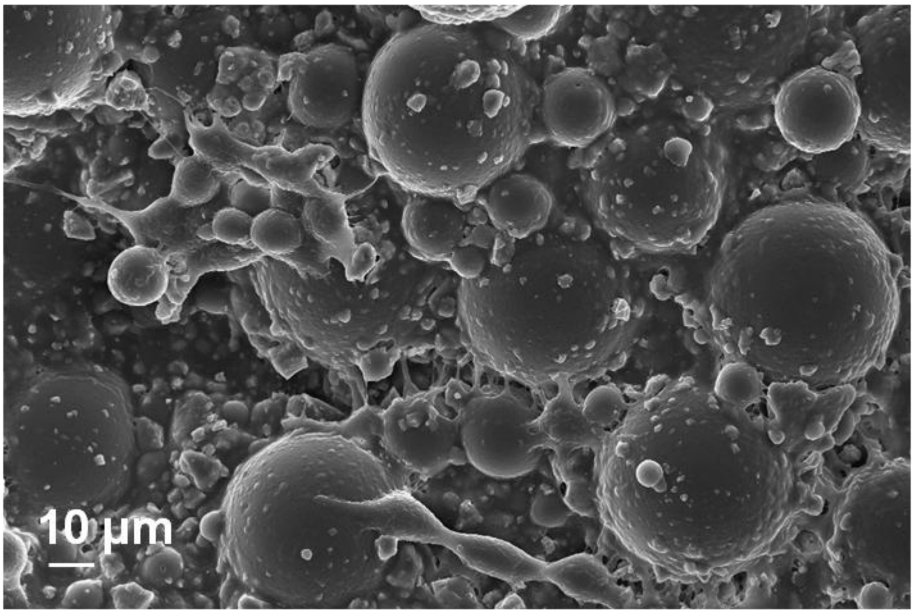


Figure 6. 91: Morphology observations of cells seeded on P20 cement after 24 hours of cultivation

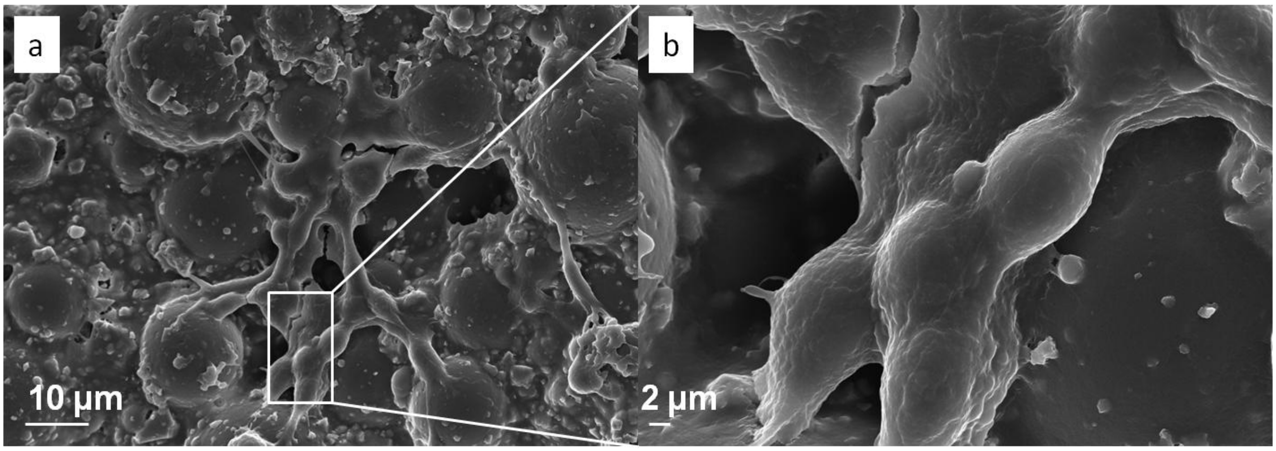
Two magnifications in figure 6.91b and 6.91c show cell adhesion on the biomaterial surface.



6. 90: Morphology observations of cells seeded on P20 cement after 24 hours of cultivation



6. 91: Morphology observation of cells seeded on P20 cement after 24 hours of cultivation



6. 92: Morphology observations of cells seeded on P20 cement after 24 hours of cultivation

Figures 6.92, 6.93 and 6.94 document some cells that create a connection one to another for the generation of new tissue only after 24 hours from seeding on the material.

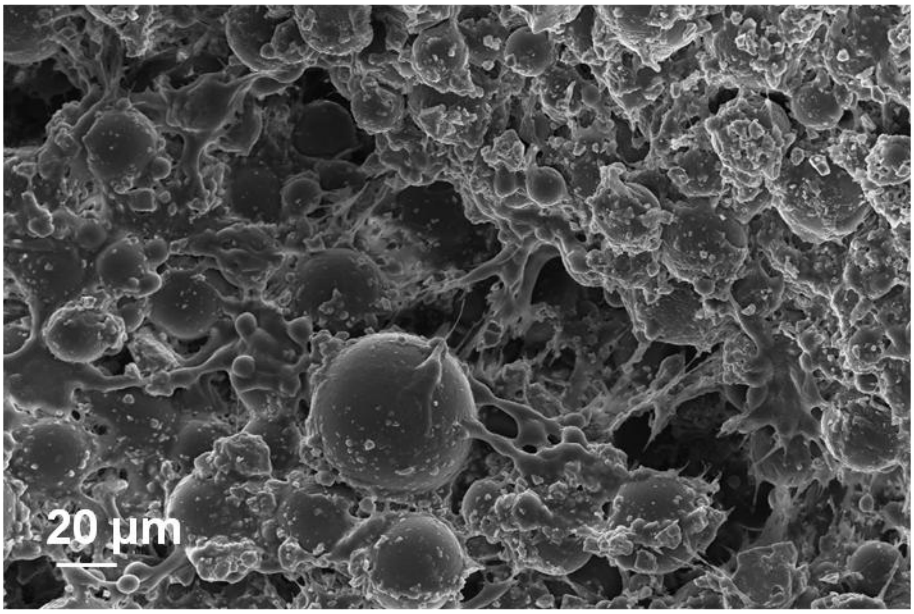


Figure 6.95: Morphology observations of cells seeded on P20 cement after 24 hours of cultivation

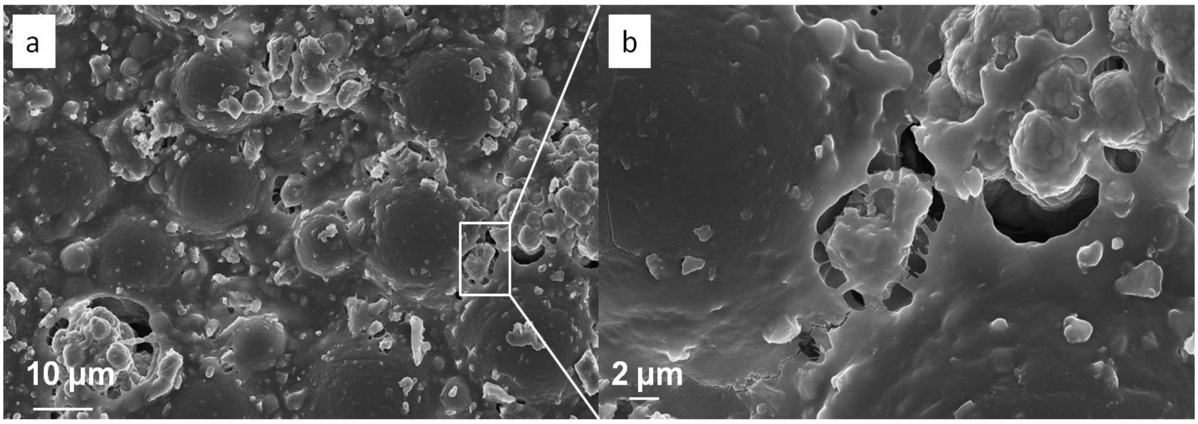


Figure 6. 96: Morphology observations of cells seeded on P20 cement after 24 hours of cultivation

Cells in figures 6.95 and 6.96 appear very well spread. They populate all the cement surface as can be seen in the magnification of figure 6.96.

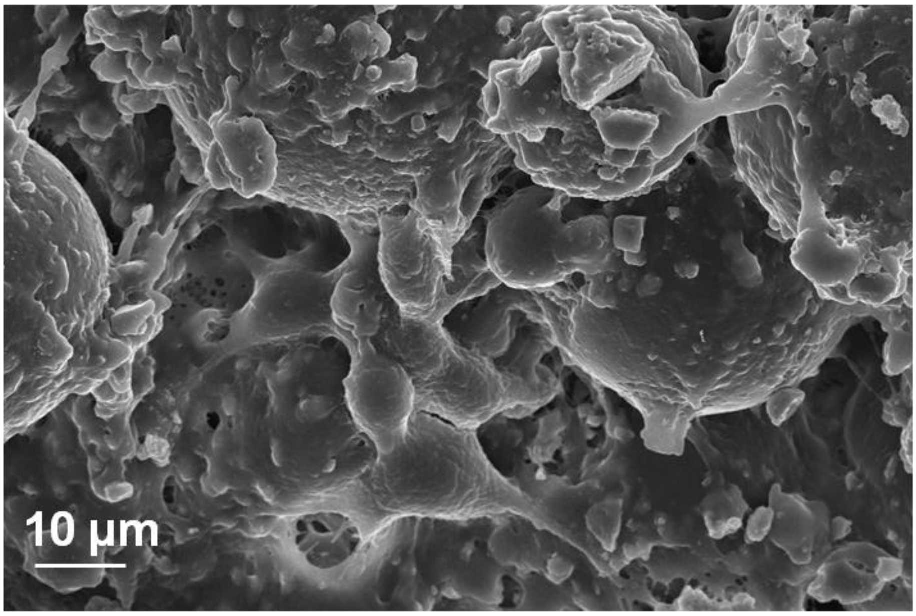


Figure 6. 97: Morphology observation of cells seeded on P20 cement after 24 hours of cultivation

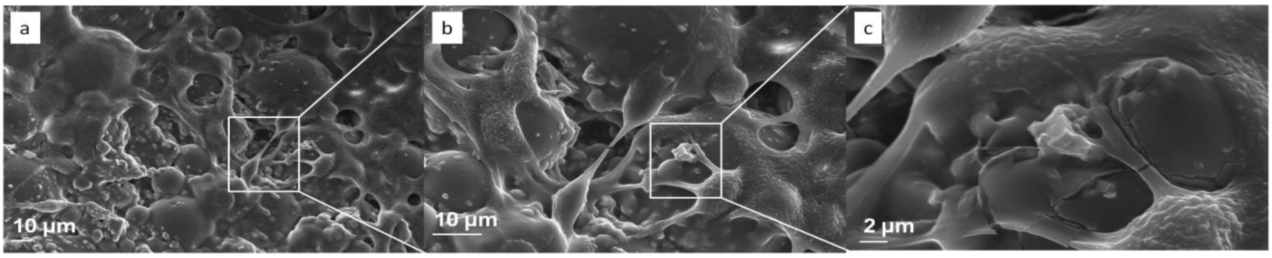


Figure 6. 98: Morphology observations of cells seeded on P20 cement after 48 hours of cultivation

After 48 hours from seeding of osteosarcoma cells, their morphology change, creating an interconnectied structure (figure 6.97). As it can be observed from figure 6.98a-b-c, cells interact more than at 24 hours and they generate a new tissue.

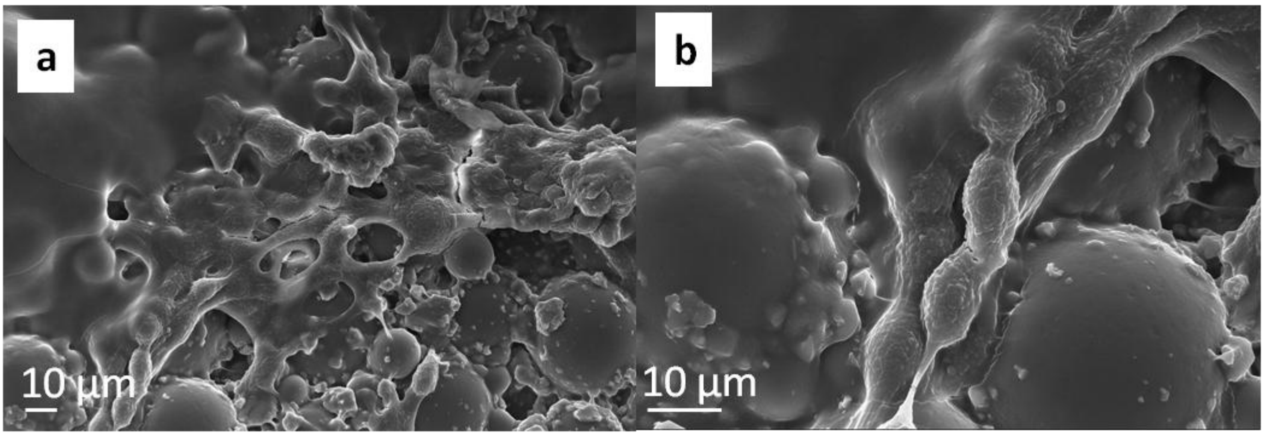


Figure 6. 99: Morphology observations of cells seeded on P20 cement after 48 hours of cultivation

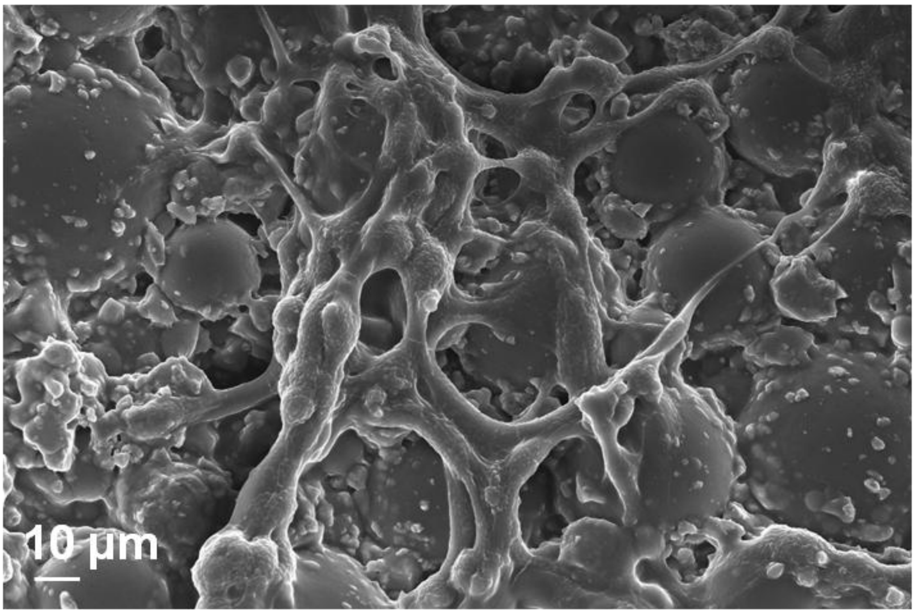


Figure 6.100: Morphology observation of cells seeded on P20 cement after 48 hours of cultivation

Analog results can be observed in figures 6.99 a-b. While in figure 6.100 cells create many bridge-like structure present in different point of P20 sample surface. This is a very important effect for positive evaluation of cytocompatiblilty of the composites.

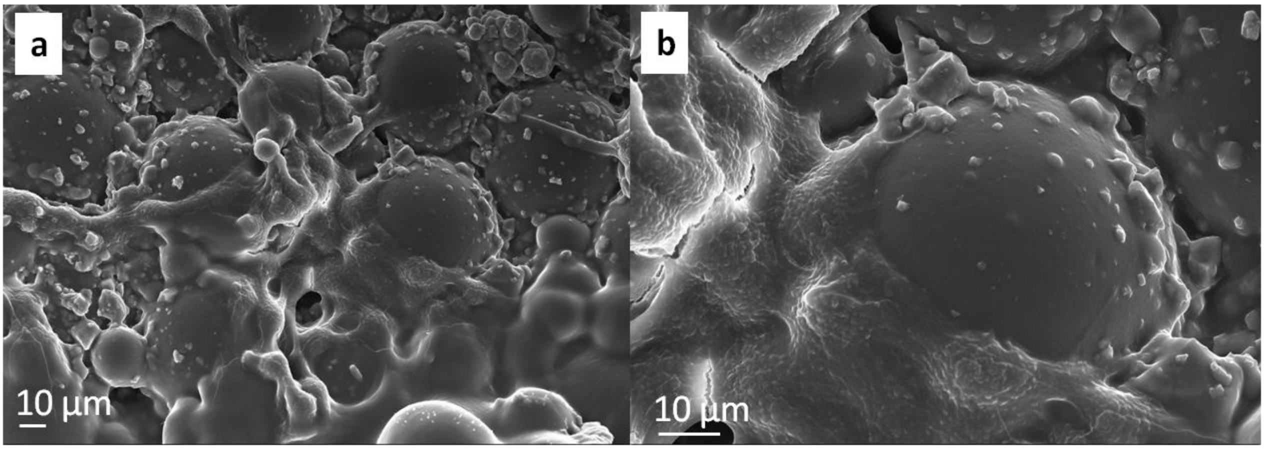


Figure 6. 101: Morphology observations of cells seeded on P20 cement after 48 hours of cultivation

In the detail of figure 6.101b several cells surround a PMMA particle . This is another example of extremely positive interaction between material and cells.

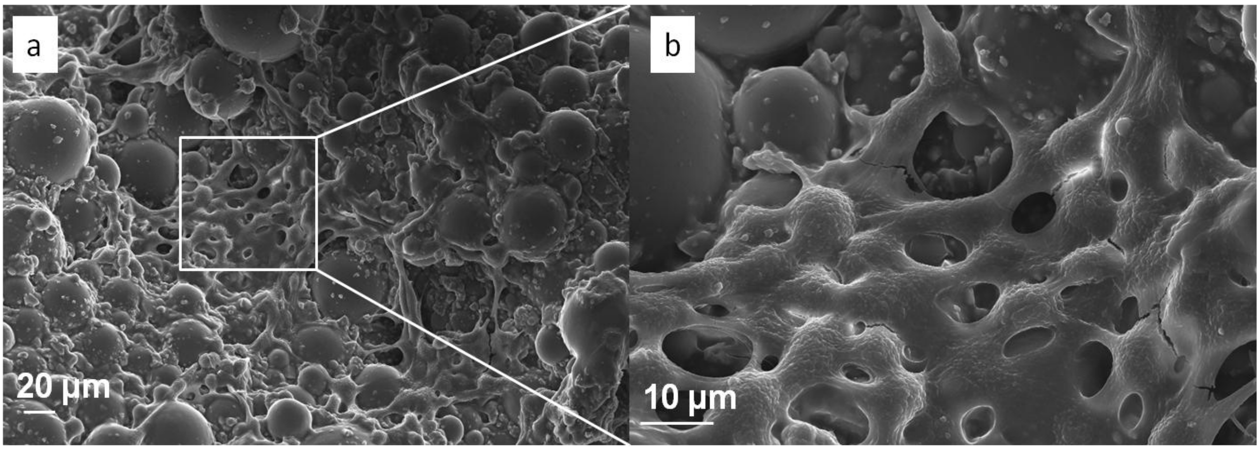


Figure 6. 102: Morphology observations of cells seeded on P20 cement after 48 hours of cultivation

Figure 6.102 reports a cover of cells that fill all available surface of the specimen. In the magnification in figure 6.102b cells create many bridge like structures and they interact for the formation of a new biological tissue.

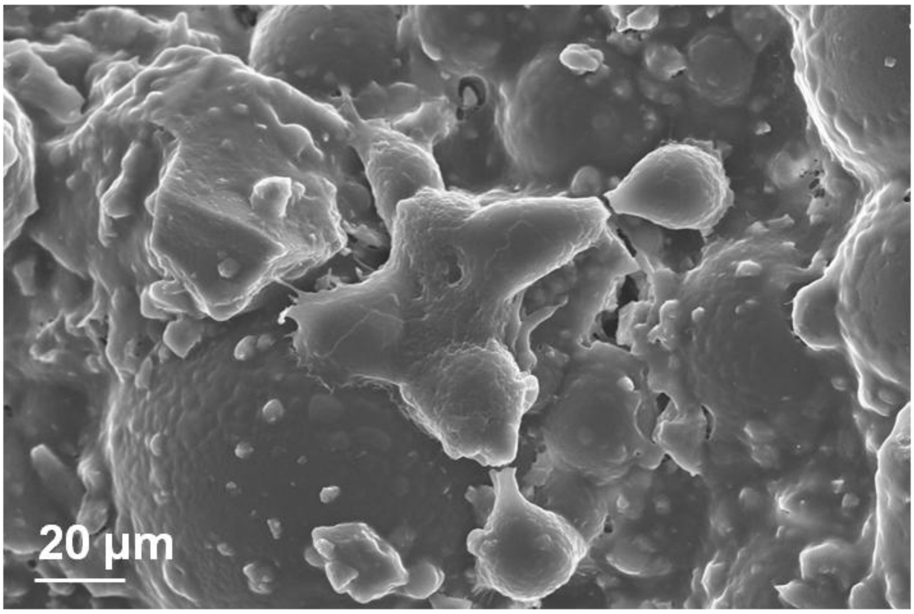


Figure 6. 103: Morphology observations of cells seeded on P20 cement after 48 hours of cultivation