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β -TCP porous scaffolds : part 2. Relation between structure and cell invasion

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Scope

- ⦿ This study sets out to explore the links between cell colonization (Osteoblasts and endothelial cells) and the porous architecture of a frozen β -TCP bone implant in vitro.
- ⦿ Shaping processes were chosen for their ability to generate original porous structures:
 - > Spherical interconnected pore network for polymeric impregnation
 - > Cubical interconnected pore network for stereolithography
 - > Ellipsoidal tubular interconnected pores for freeze casting
- ⦿ Samples obtained by impregnation of a polymeric skeleton will serve as comparison standard.



The manufacturing of **β -TCP powder** was achieved through aqueous precipitation using diammonium phosphate and calcium nitrate solutions.

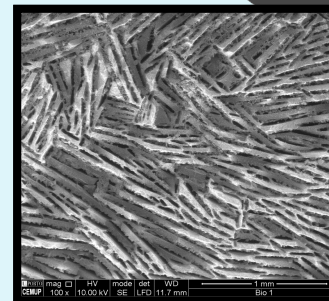
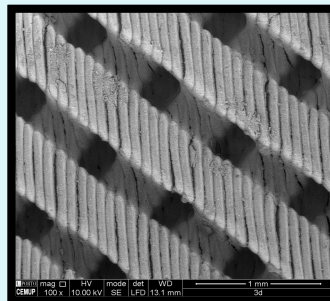
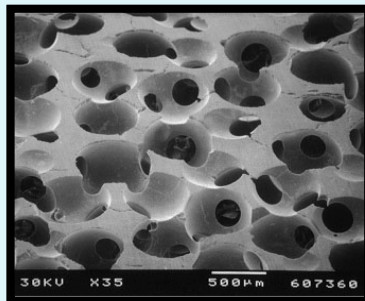
β -TCP shaping processes

Impregnation of a polymeric skeleton (**PS**)

Stereolithography (**3D**)

Freeze casting (**BIO 1, 4 and 7**)

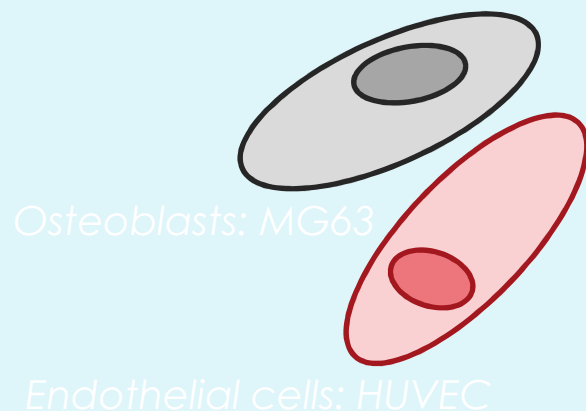
Sintering (1100°C / 3 h)



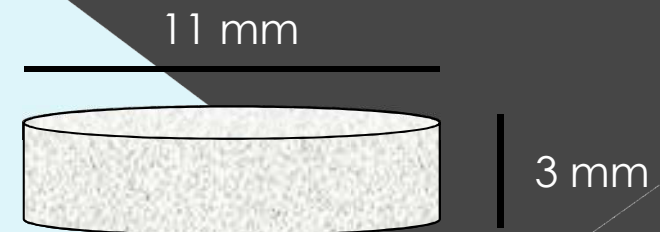
Cell culture

- Sample characteristics:

Sample denomination	Shaping method	Porosity (%)	Pore and interconnection diameter (μm)
3D	stereolithography	50	500 / 100
PS	Impregnation of a polymeric skeleton	65	400 – 500 / 100
BIO 1 BIO 4 BIO 7	Freeze casting	50 50 36	150 / 40 360 / 55 150 / 45



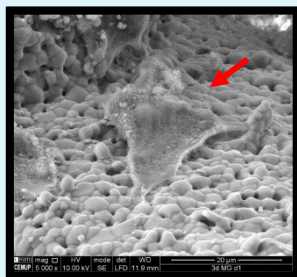
2×10^5 cells / well



Sample dimensions were defined according to 24 wells culture plates dimensions

- All samples were incubated without cells for various periods of time and then seeded with osteoblasts (MG63) in order to determine incubation duration:

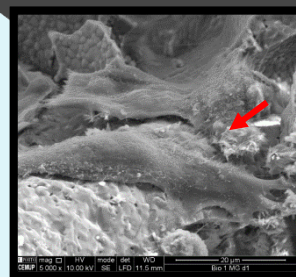
Incubation period (h)	Observations
1	High cell mortality : no cell survival past Day 3
5	High cell mortality : no cell survival past Day 3
24	High cell survival: positive response noticed



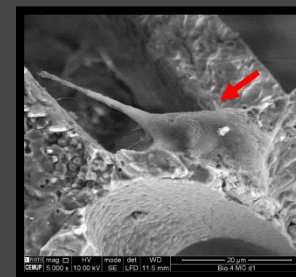
3D



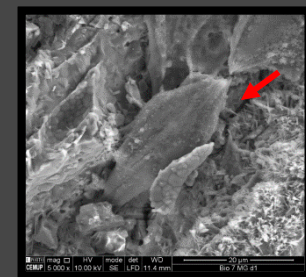
PS



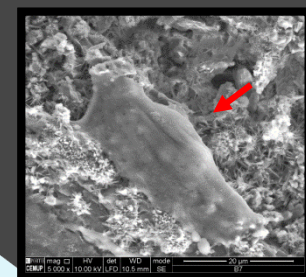
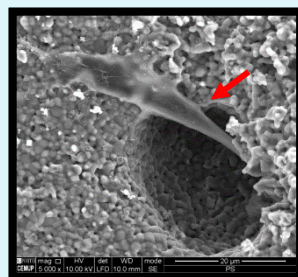
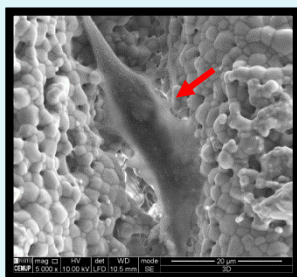
Bio 1



Bio 4



Bio 7



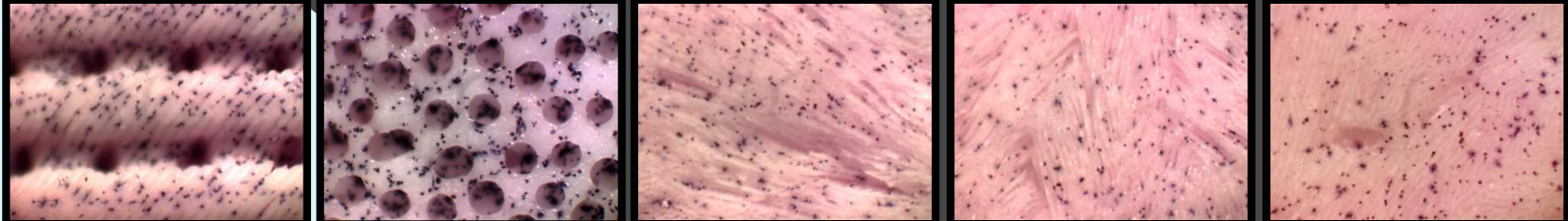
SEM pictures taken at Day 1 and 3 (post seeding), after 24 h of incubation with culture medium. High magnification (x 5000) allowed morphological examination of cells

QM and SEM observations – DAY 1

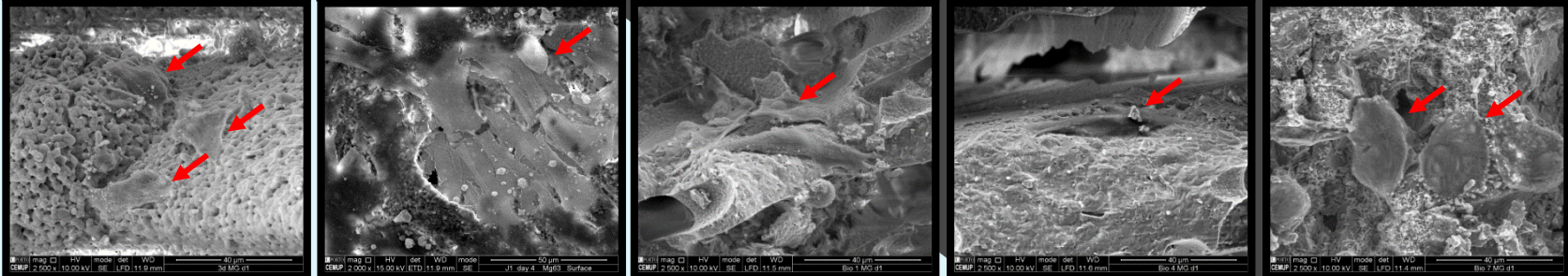
10x



35x



2500x



3D

PS

Bio 1

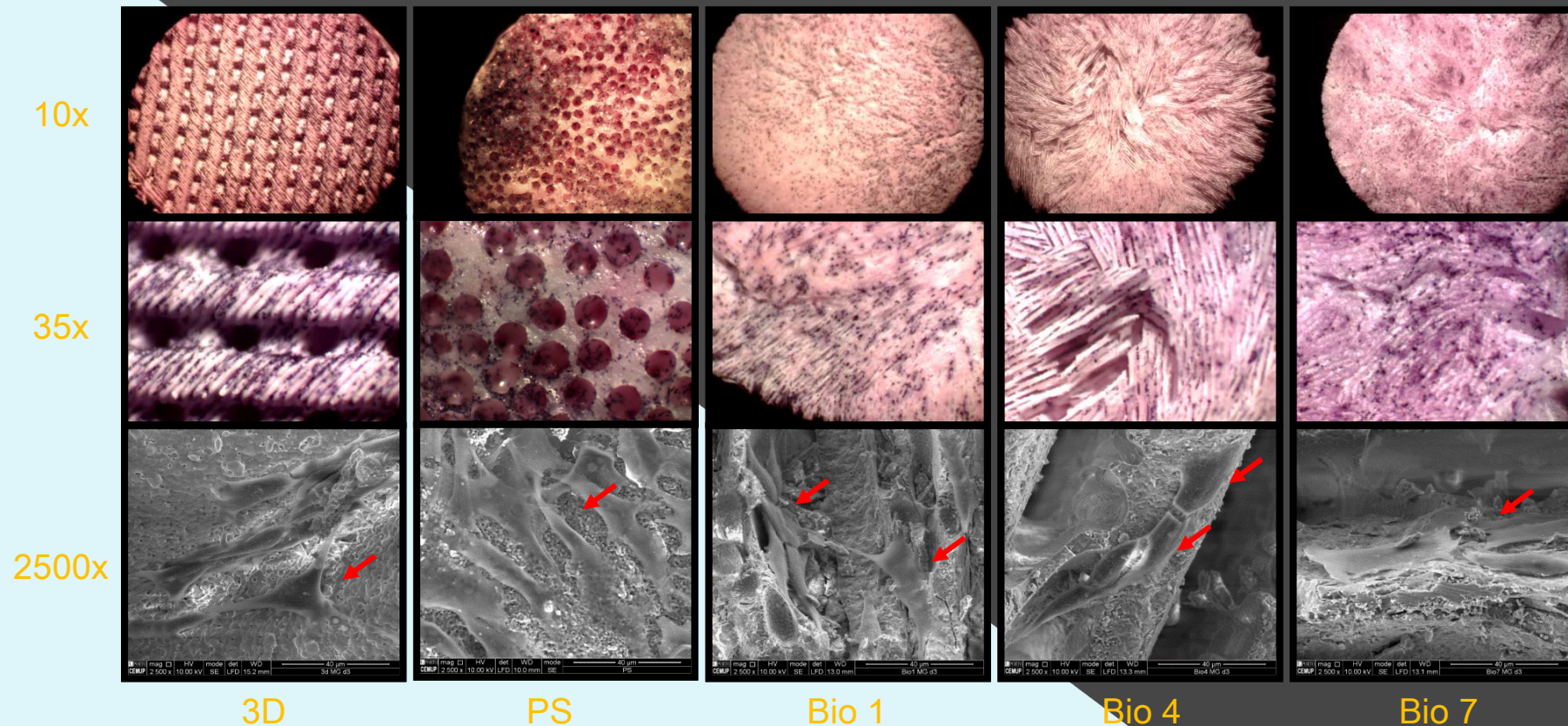
Bio 4

Bio 7

MG63: Osteoblasts responded well. MTT stained cells can be seen all over the samples. Their morphology suggest that they are adherent to the material.

HUVECs: Endothelial cells responded poorly. Control well seems to indicate that the material is not responsible for this response. (Results not shown)

QM and SEM observations – DAY 3



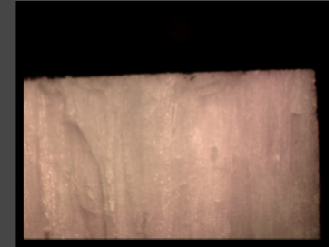
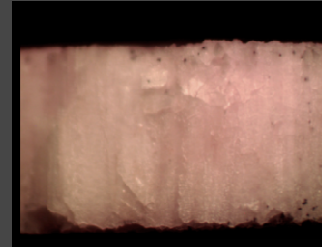
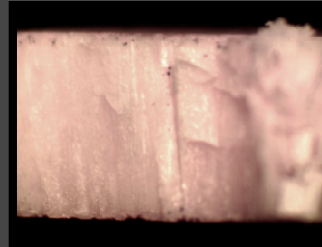
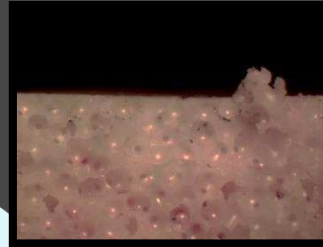
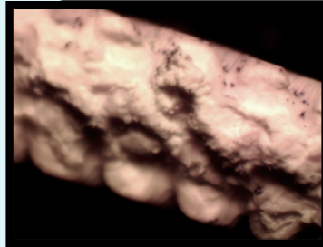
MG63: cell population increased on all the samples. However, 3D and PS samples seem to have a denser cell population.

HUVECs: No endothelial cells were observed. (Results not shown)

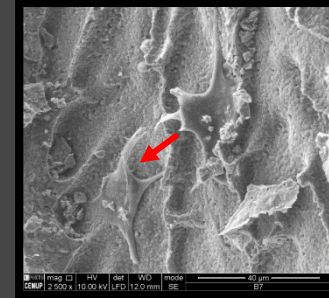
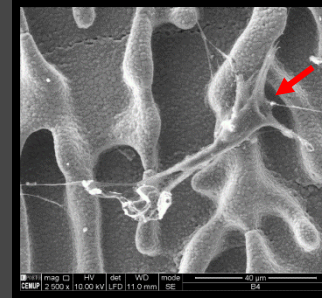
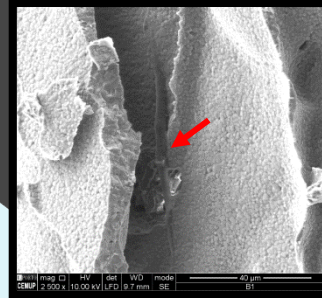
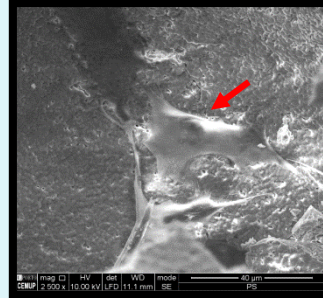
Cell penetration – DAY 1

Day 1

x 20



2500x



3D

PS

Bio 1

Bio 4

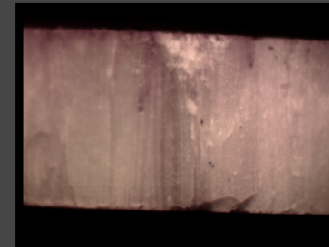
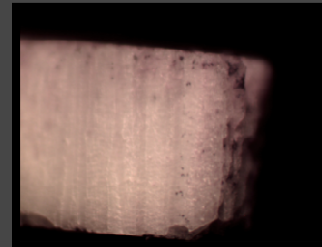
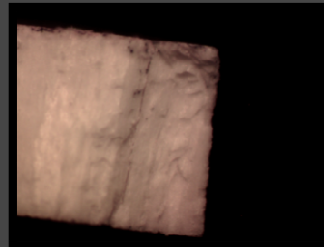
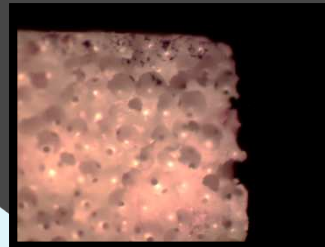
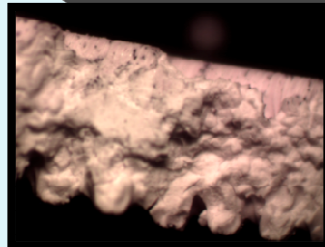
Bio 7

MG63: cell penetration seems to be limited to the areas closest to the surface. SEM pictures show flattened cells with cytoplasmic extensions.

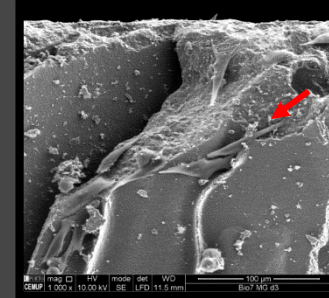
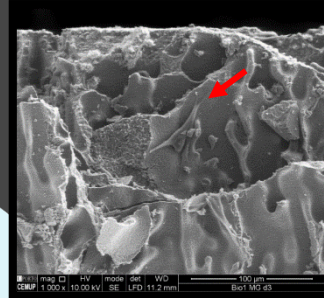
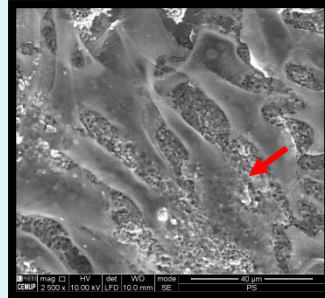
Cell penetration – DAY 3

Day 3

x 20



2500x



3D

PS

Bio 1

Bio 4

Bio 7

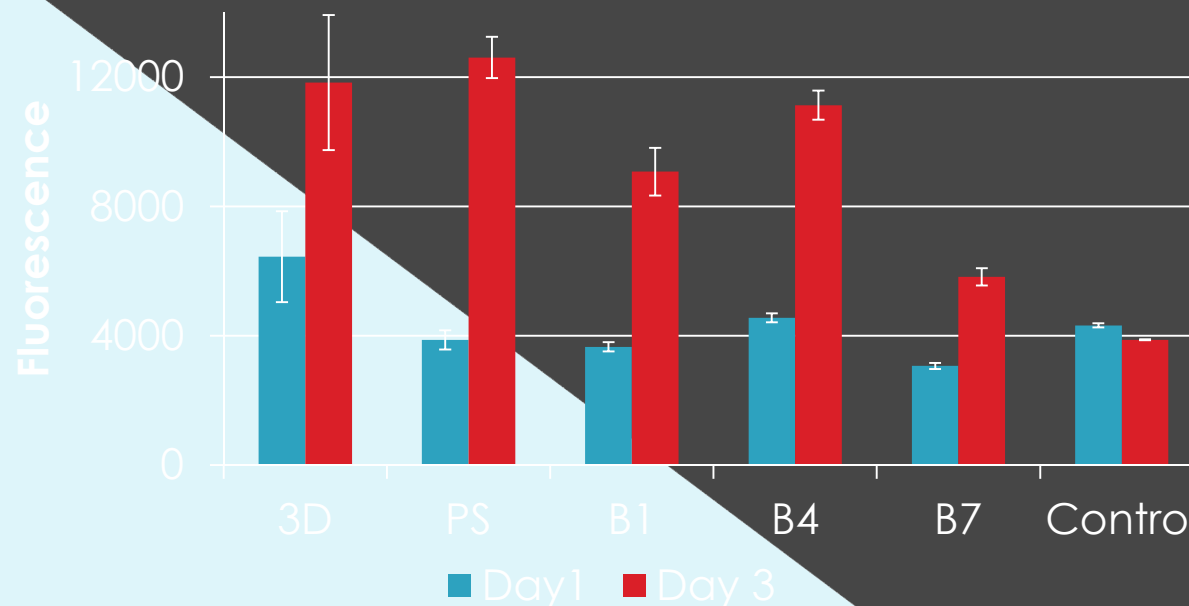
MG63: cell populations found inside the samples were larger than on the first day. Their morphology suggest that they were adherent and well adapted.

Cell penetration in PS and 3D samples is restricted to the pores closest to their surface whereas frozen sample had cell deep into their porous network (Bio 4).

Global porosity does not seem to have an impact on cell penetration.

Resazurin Assay

Resazurin Assay: MG63

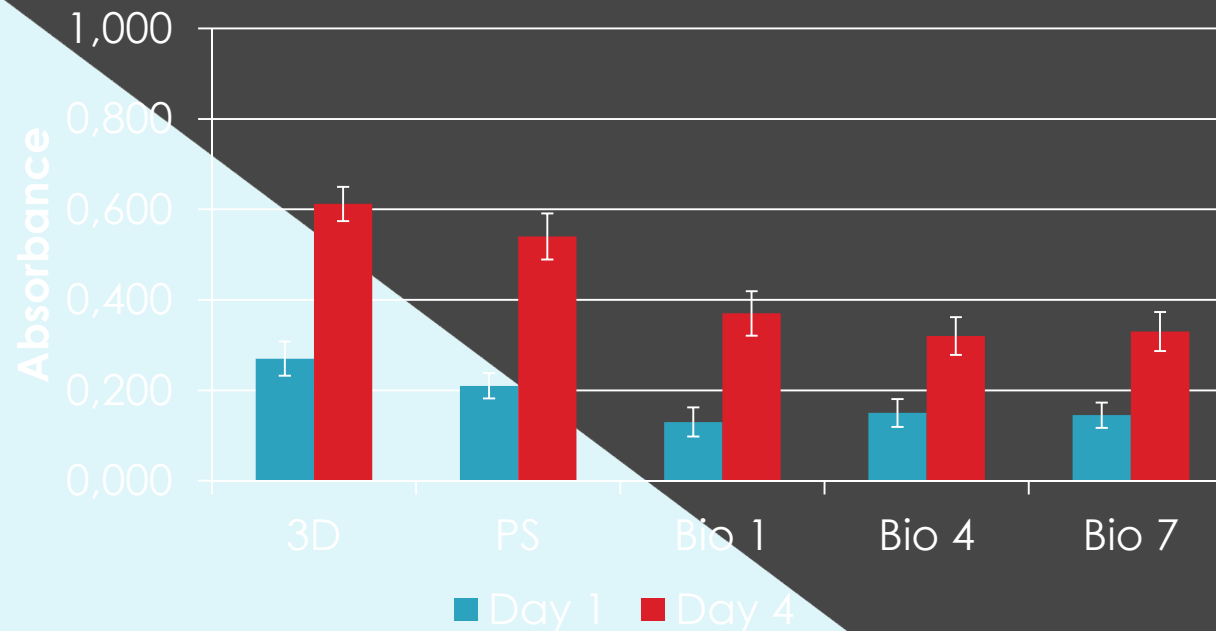


Control: unseeded well with material

Resazurin assay confirmed the conclusions reached after observation of samples. Osteoblasts performed well with a steady increase of their population from day 1 to 3. Among the frozen samples, Bio 4 shows better performances.

MTT Assay

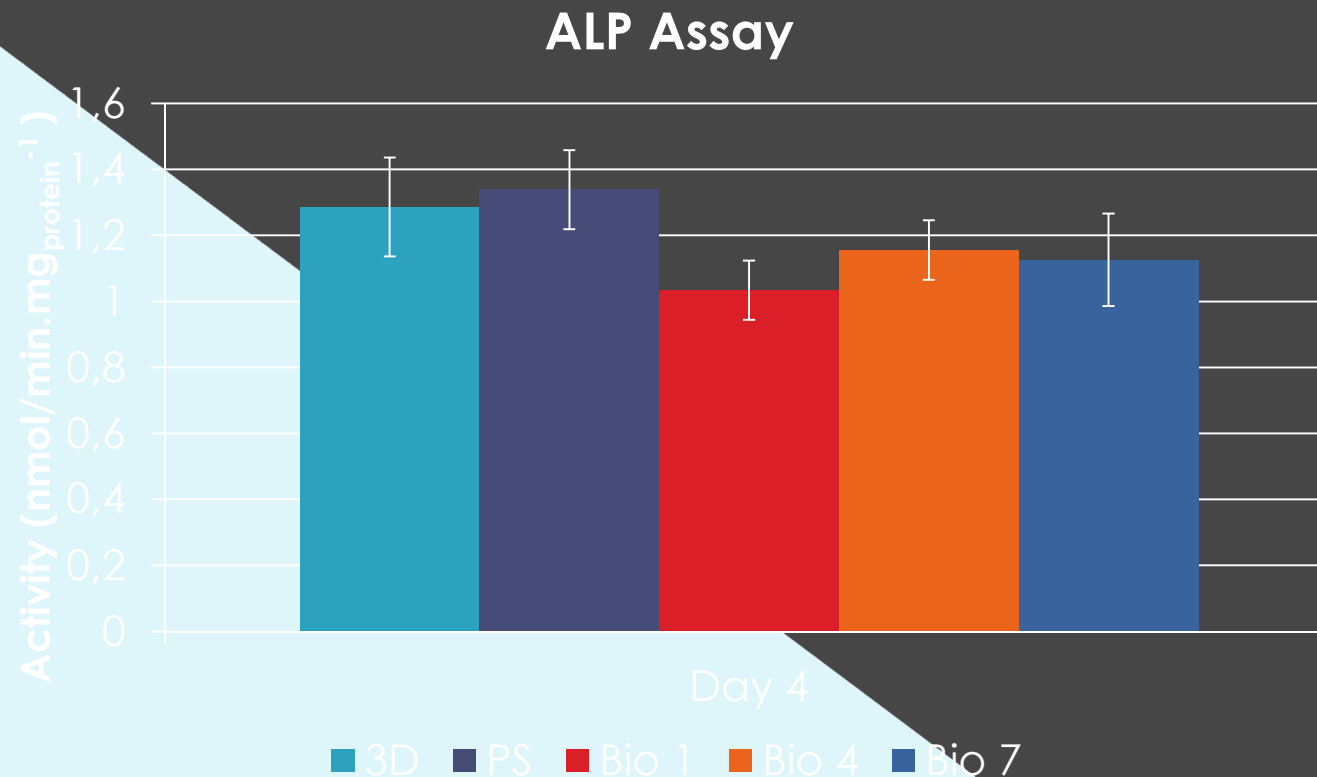
MTT Assay : MG63



Control: unseeded well with material

An MTT assay shows that 3D and PS samples contain more cells than the frozen samples. frozen samples show no significant difference between each other.

Alkaline Phosphatase Assay



Activity was normalized to the total protein content

Levels of ALP activity are very similar. ALP activity seems to be slightly higher in PS and 3D samples. However the levels are so close that it can be said that ALP is as active in frozen samples as it is in 3D or PS samples.

Conclusion

- Shaping techniques allowed the fabrication of samples exhibiting original structures.
- Bioactivity of β -TCP did not seem to be altered by shaping processes
- Osteoblasts reacted well to the material (Flat adherent cells with cytoplasmic extensions)
- 3D and PS samples hosted the largest number of cells at day 4 (MTT Assay).
- Cells penetrated further into the frozen samples (Bio 4), it could be explained by the presence of the local tubular structure and its diameter.
- Frozen samples (Bio 1, 4 and 7) exhibited levels of alkaline phosphatase activity as high as in 3D and PS samples.

Outlooks

- These preliminary tests and results highlighted several issues and allowed for the fine tuning of the cell culture protocols for future experimentation.
- The next step of this study will be to perform the above tests over longer periods of time (7 to 21 days).
- Future tests: endothelial cell culture, co-cultures, gene activity (RT-PCR), in vivo tests...

Acknowledgements



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