Techniques to determine cell adhesion

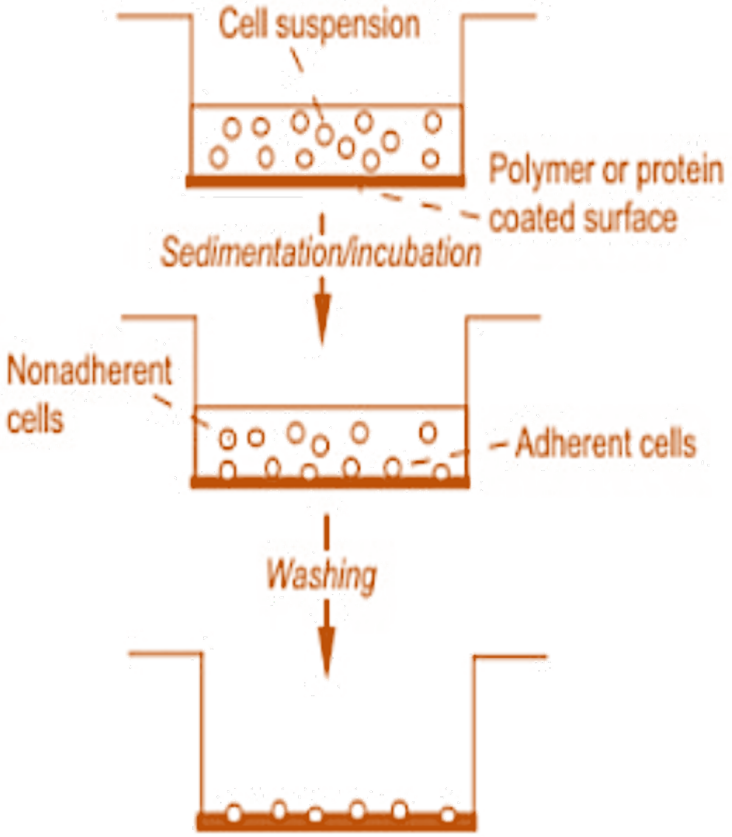
- Sedimentation-detachment assay

i) sedimentation of cells onto a surface

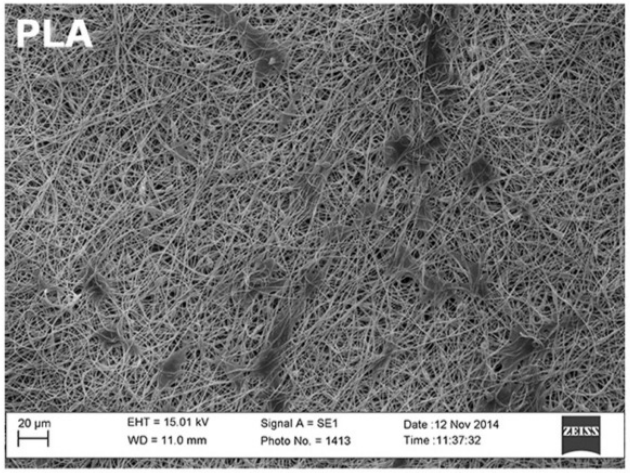
ii) incubation of the sedimented cells in culture medium for some period of time

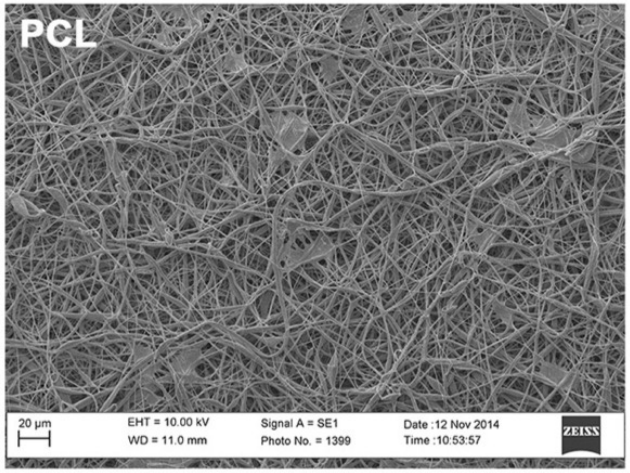
iii) detachment of loosely adherent cells by removal of the culture medium and repeated washing

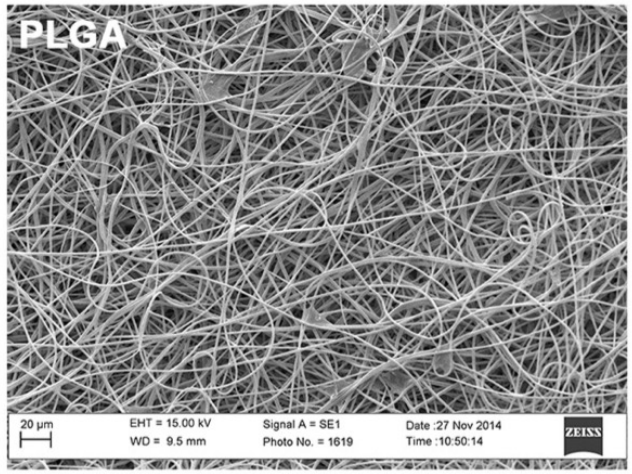
- The extent of adhesion is determined by the number of cells that remain associated with the surface or the number of cells that were extracted with washes.

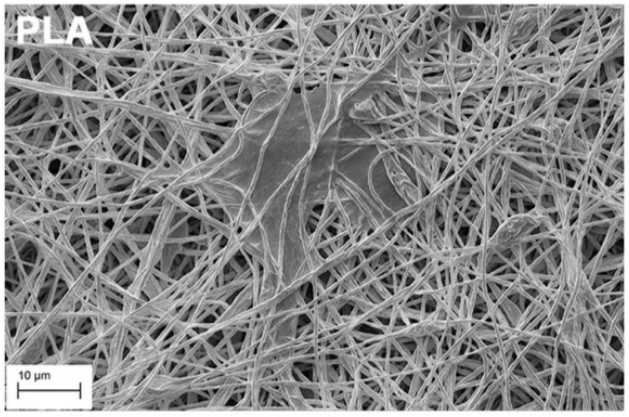


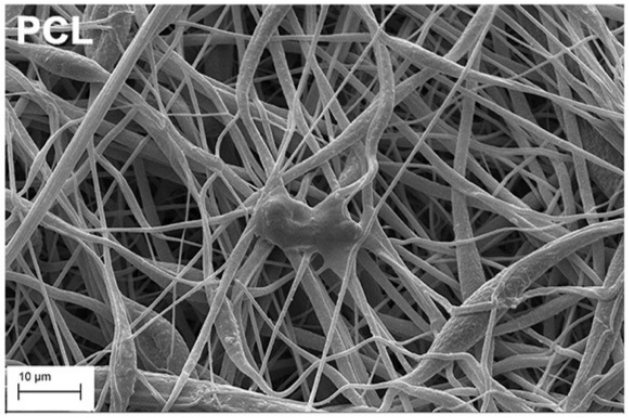
Cell engraftment on scaffolds: Scanning Electron Microscope ultrastructural analysis.

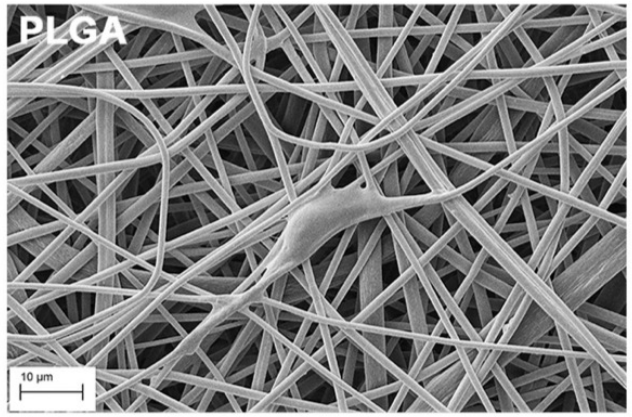




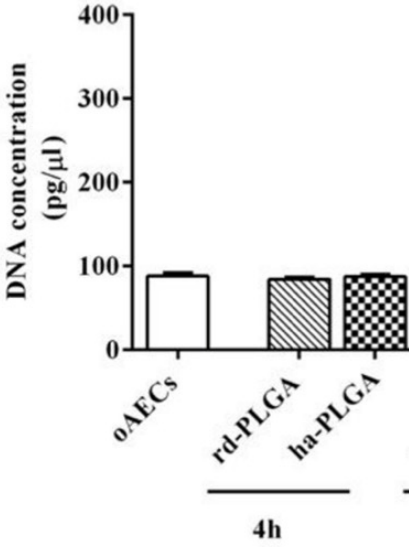








DNA Quantification: cell seeding, adhesion efficiency



- Amount of DNA in a sample gives indication of cell growth on the scaffold.
- This can be assessed Quantitatively and qualitatively.
- Quantitative- by **Real time PCR** analysis, which directly measures amount of target gene in the sample.
- Qualitatively- using Hoechst dye (for labelling DNA) followed by **fluorometric analysis** to yield count of cells showing fluorescence.

Quantification of fluorescently labelled cells

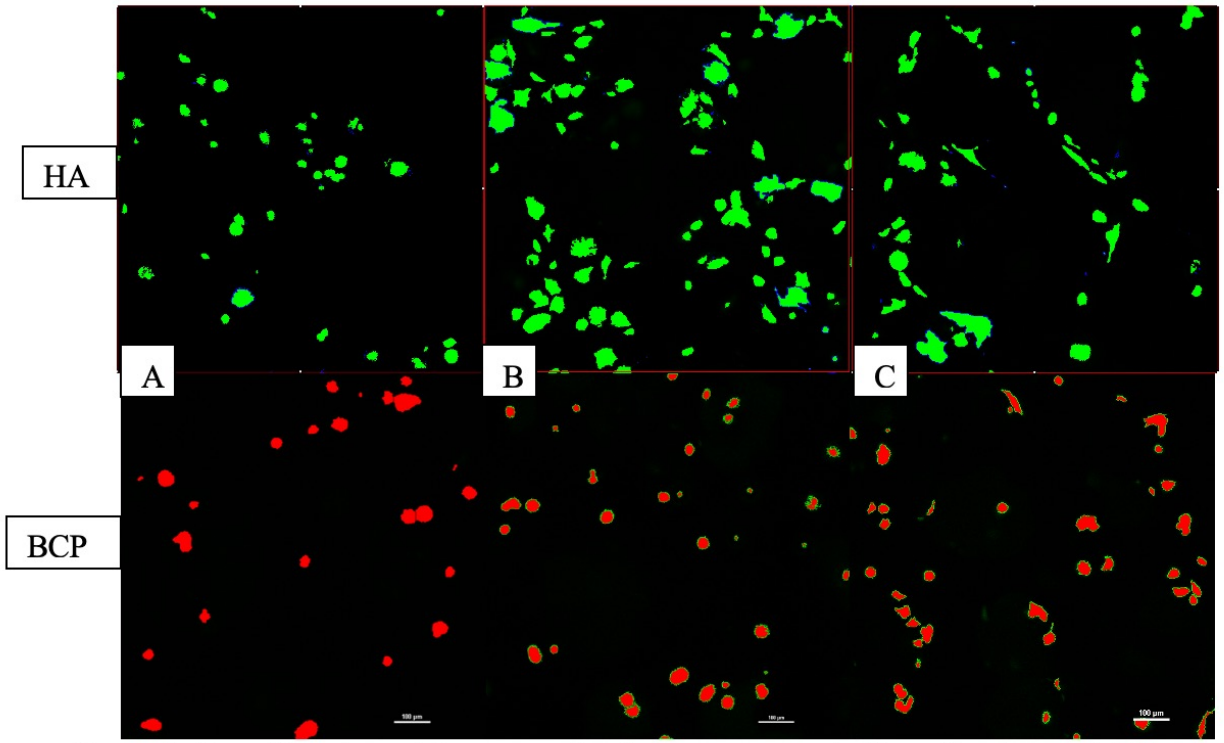
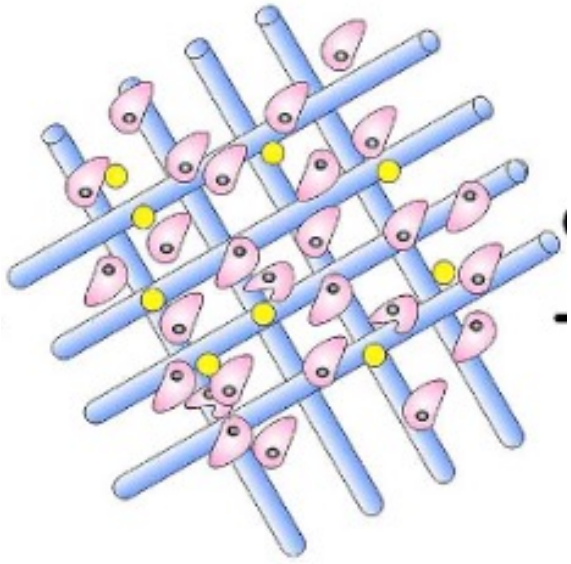


Figura 1. Quantificazione del numero di cellule per area nota (1mm^2) su immagini binarizzate ottenute da colture cellulari con entrambe le tipologie di scaffold a diverse concentrazioni (A) 2,5 e (B) 5 e (C) 10 x 10^5.

Concentrazione	2,5 X 10^5	5 X 10^5	10 X 10^5
Numero di cellule per mm^2	46±2	83±4*	51±3

Distribution and Spreading



Distribution

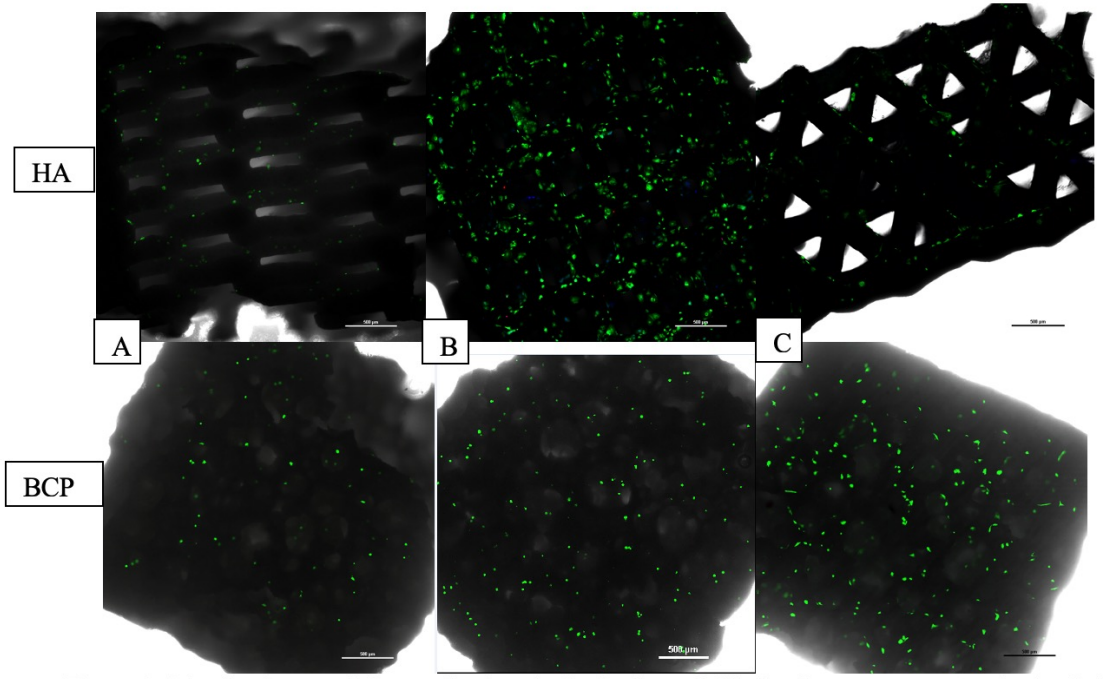


Figura 2. Distribuzione cellulare sulle due tipologie di scaffold alle diverse concentrazioni cellulari (A) 2,5 e (B) 5 e (C) 10 x 10^5. Le cellule sono state colorate con calceina AM (verde); propidio (rosso) e Hoechst (blu).

Cell Spreading

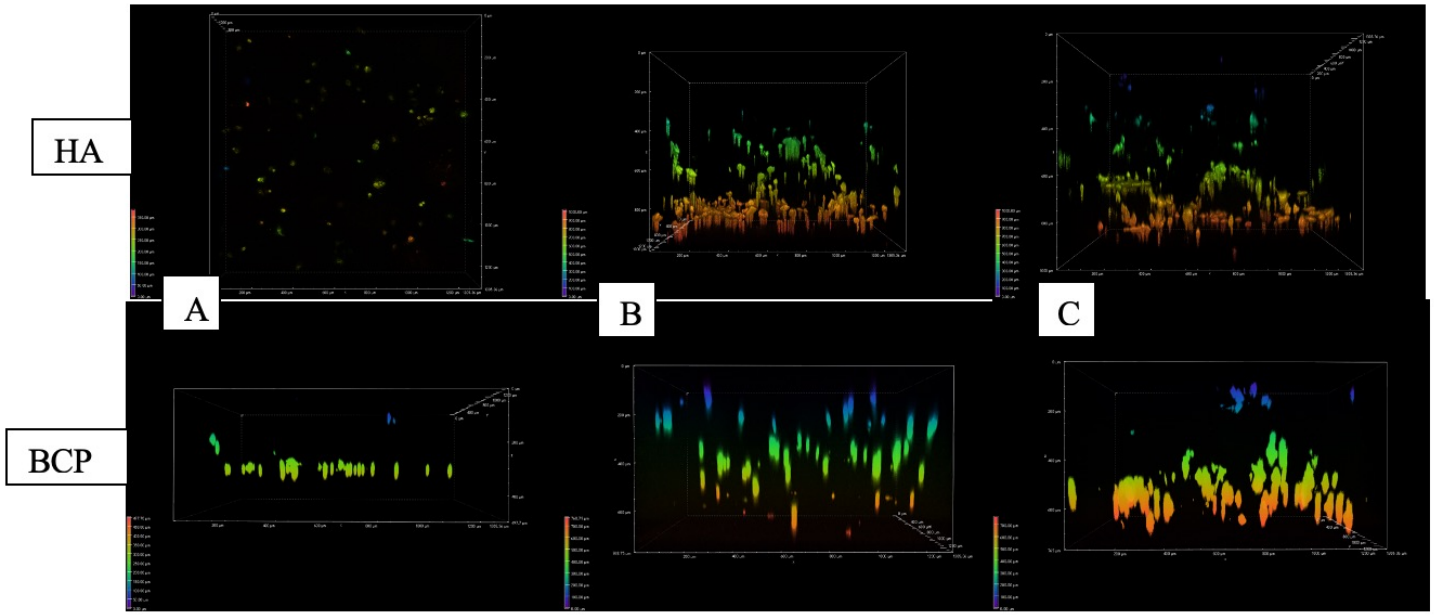


Figura 3. Gradiente di penetrazione cellulare all'interno degli scaffold HA bifasico e BCP alle diverse concentrazioni cellulari (A) 2,5 e (B) 5 e (C) 10 x 10^5.

Cell penetration: Depth Coded Maximum projection analysis