

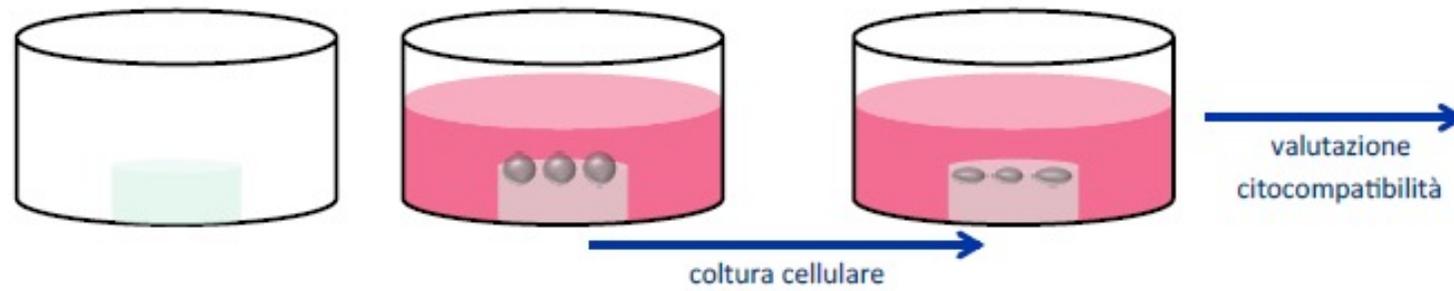
# Biocompatibility

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- The ability of a material to elicit an appropriate biological response in a specific application by **NOT** producing a toxic, injurious, or immunological response in living tissue.

- **Biocompatibility testing:**
- *Invitro*- SEM, Fluorescence microscopy → Cell morphology, attachment and spreading
  - Alamar blue assay → Cell proliferation
  - MTT assay → cell viability and metabolic activity analysis
- *Invivo*- preclinical tests in animal models- creating artificial wound and accessing the response generated by the implant.
- Next step is clinical trial- performed by surgeons in hospitals

# Direct Biocompatibility



# Valutazione

**Qualitativa:** visualizzare cellule per confermarne la presenza, l'adesione e la morfologia attraverso diverse tecniche di microscopia:

- Osservazione della morfologia cellulare
- SEM

**Semi-quantitativa:**

- Microscopio a fluorescenza o confocale:
  - LIVE/DEAD (a seconda del reagente) le cellule vive/verdi o morte/rosse
  - Proliferazione cellulare

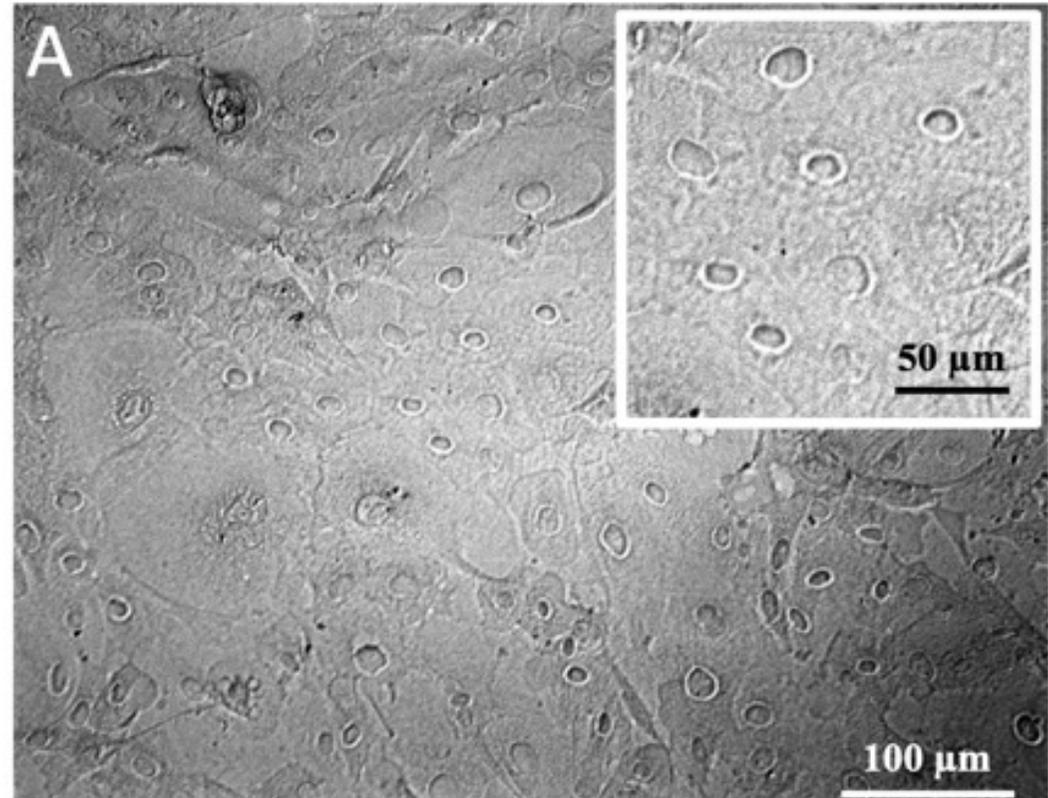
**Quantitativa:**

Proliferazione cellulare:

- Saggi MTT e Alamar Blue (metodi indiretti)
- Biologia Molecolare

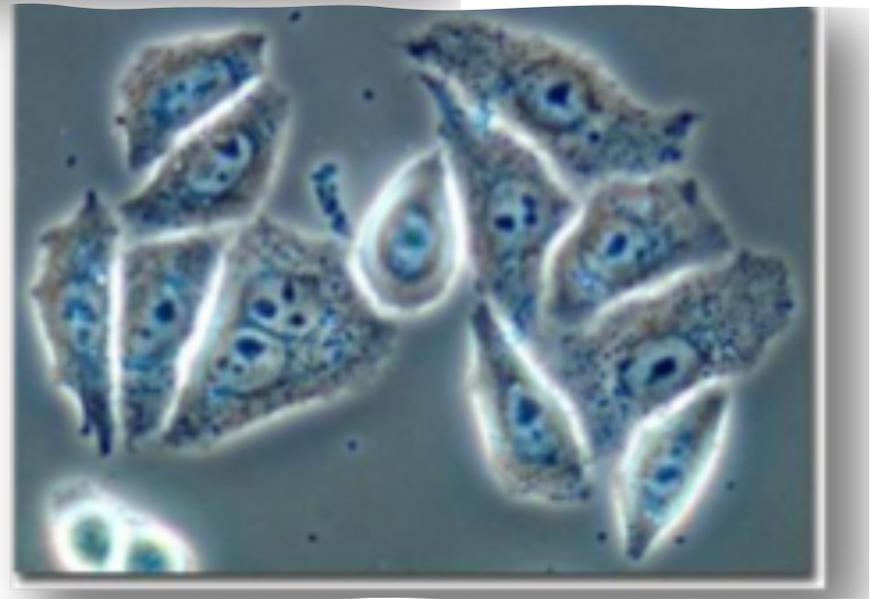
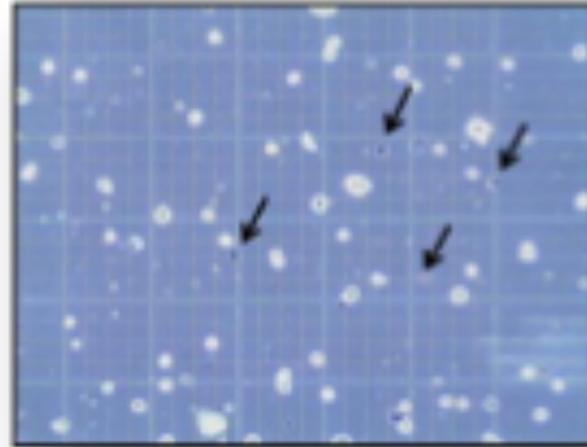
Osservazione  
della  
morfologia  
cellulare

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# Trypan blue

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# LIVE/DEAD ASSAY

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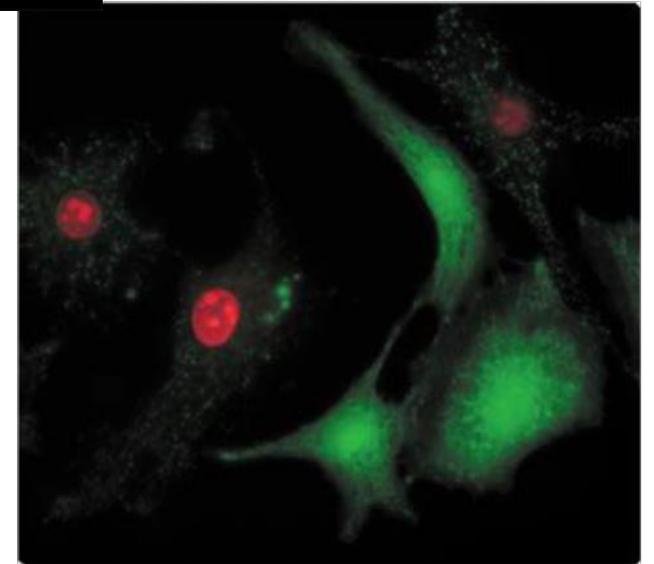
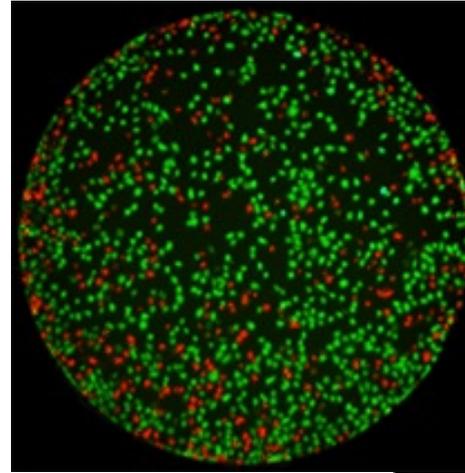
Then various dyes are used to stain different cellular components. These are then examined under Fluorescence or confocal microscope to yield images according to stains used.

Dye name	Stains
Calcein-AM	Live cells
EtBr (Ethidium Bromide)	Dead cell nucleus
Phalloidin	Cell cytoskeleton
DAPI	Nucleus of live cells
Propidium Iodide (PI)	Dead cell staining
Hoechst	Nucleus of live cells
MitoRed	Mitochondria

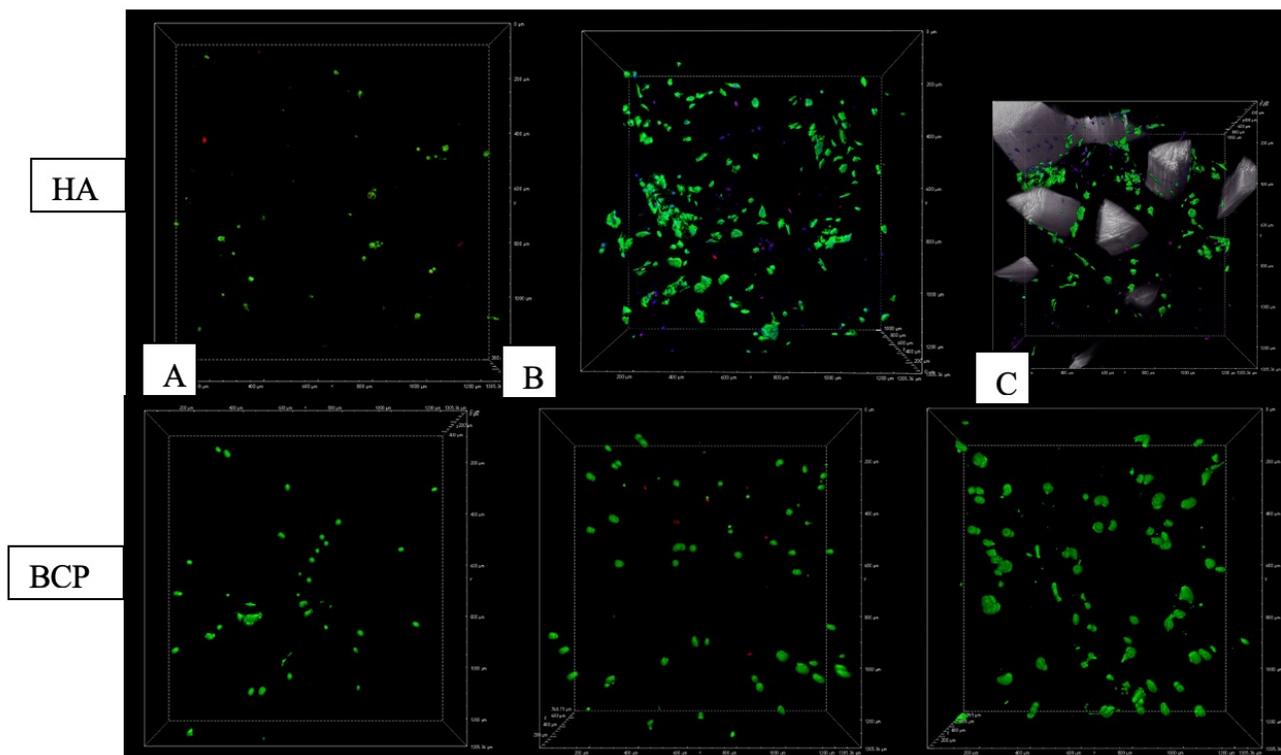
# Cell viability: Calcein AM/PI

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Calceina AM (acetometossicalceina) = verde  
Propidio Ioduro = rosso

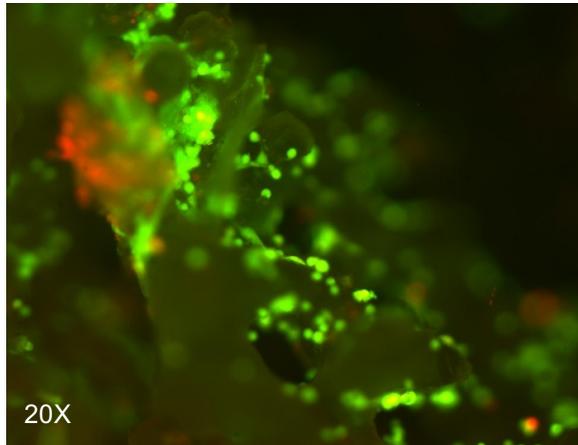


# Calcein AM/PI



# Biocompatibility

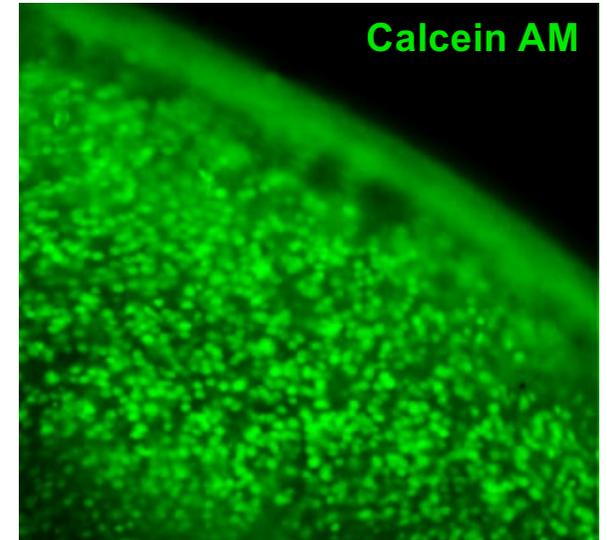
## Cell viability



AEC / scaffold

Calcein AM = alive cells

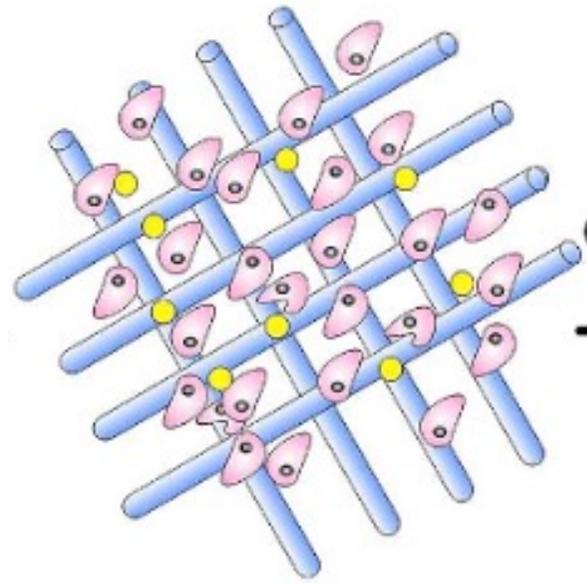
Propidium iodide = dead cells



### Cell viability (%alive cells/ total cells)

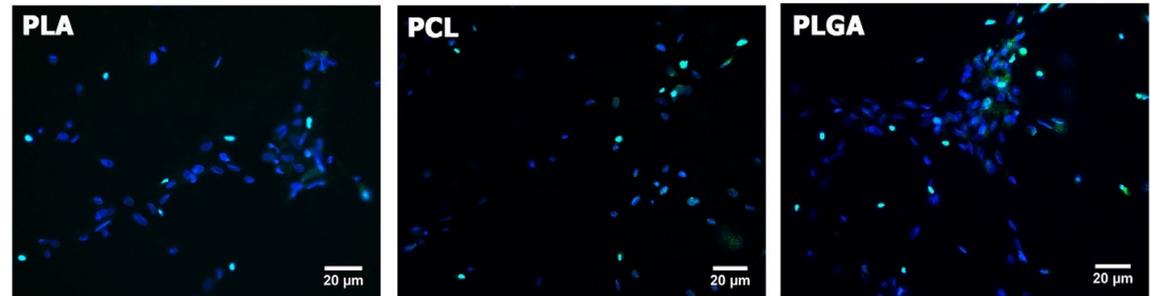
AEC

88%

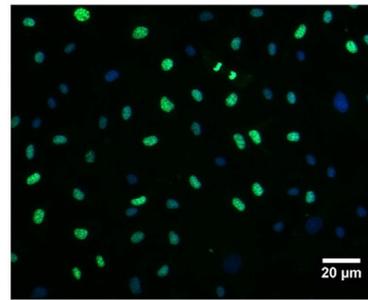


Cell proliferation

# Proliferazione cellulare: saggio semi-quantitativo

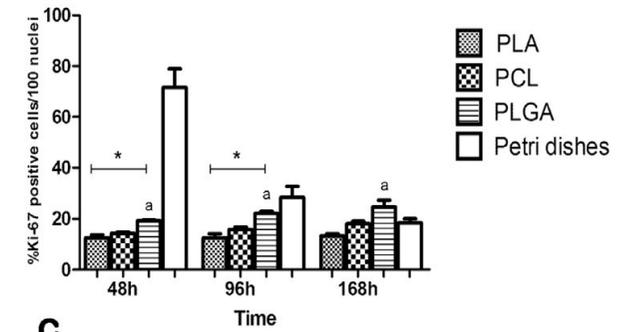


**A**

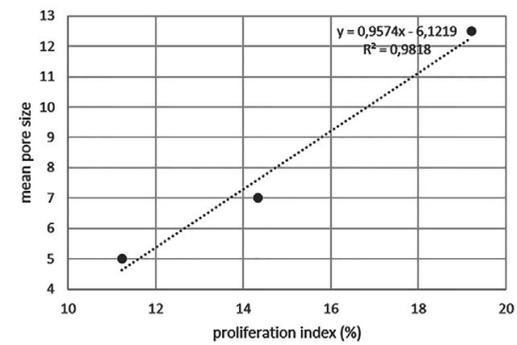


**B**

oAEC Proliferation Index



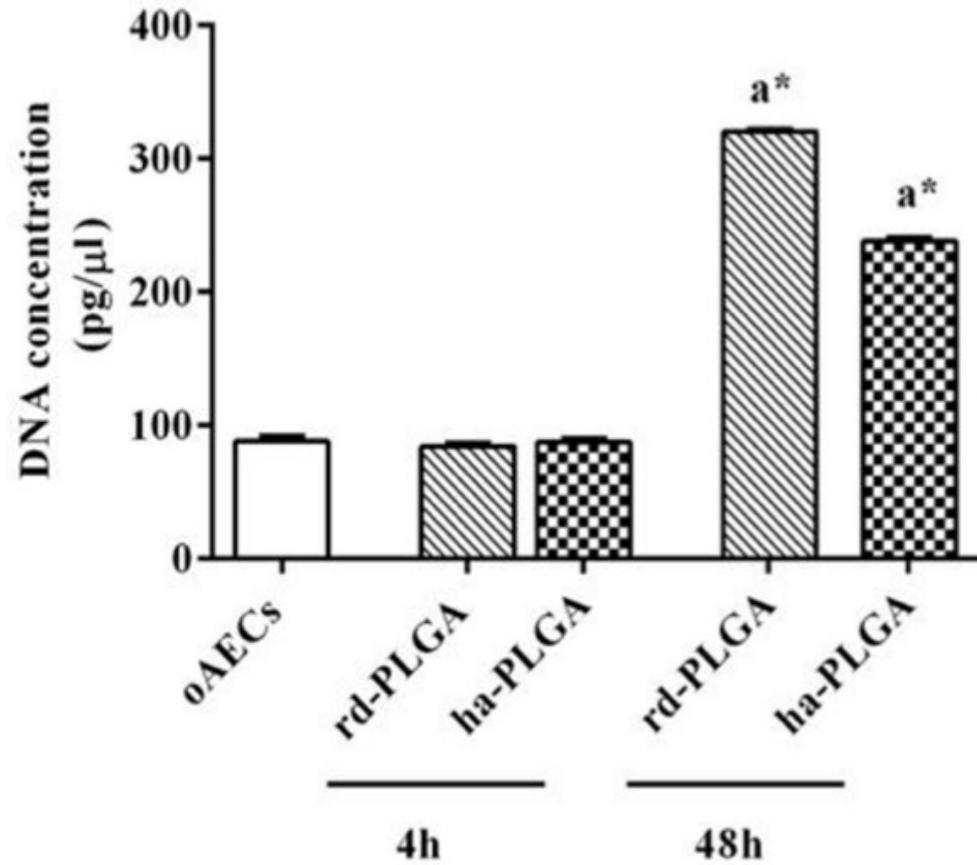
**C**



**D**

# Proliferazione cellulare: saggio quantitativo

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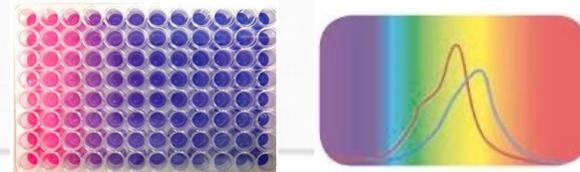
# Alamar blue assay

## Proliferazione cellulare: saggio quantitative indiretto

### Principle:

Alamar Blue Reagent is a non-toxic, water-soluble resazurin dye that yields a fluorescent signal and a colorimetric change when incubated with metabolically active cells. Absorbance at 570nm and 600nm yields cell proliferation rate.

### Process:



Measure Absorbance (570nm and 600nm)



# MTT Assay: saggio quantitativo indiretto

## Principle:

The yellow tetrazolium MTT is reduced by metabolically active cells, by the action of dehydrogenase enzymes, to formazan. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometry.

## Process:

- Small disks of scaffolds seeded with cell suspension ( $\sim 4 \times 10^5$  cells/ml approx).
- After incubation, MTT solution (5 mg/ml) is added to the culture well and incubated for 4hr. Colour changes from yellow to purple.
- Then purple colour precipitate of Formazan is solubilized by adding Dimethyl sulfoxide (DMSO).
- The mitochondrial activity is measured by taking spectrophotometric reading at 595nm.

