Distinct polymeric based materials prepared/functionalized by gamma irradiation for biomedical applications and Roman mosaics preservation

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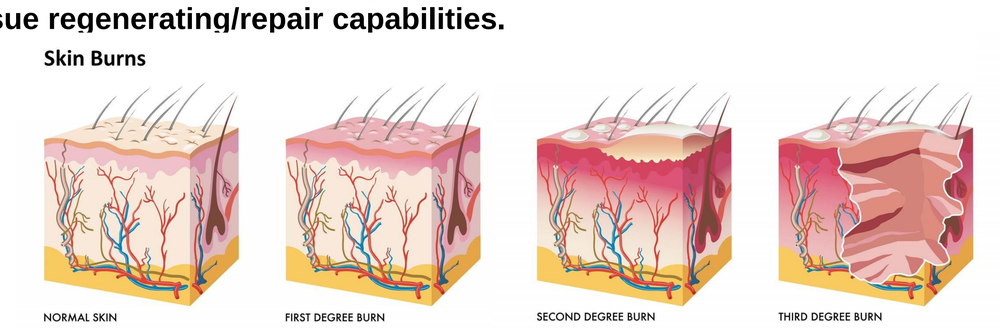
Chitosan based copolymers

Motivation:

Create biocompatible and biodegradable skin substitutes with improved healing and tissue regenerating/repair capabilities.



Objective:

Optimize the production and sterilize three-dimensional biocompatible and biodegradable chitosan based matrices by gamma irradiation, for use as skin scaffolds.

PDMS based hybrid materials

Motivation:

Efficient preservation of Roman mosaics in the most important Portuguese archaeological site – *Conimbriga*.

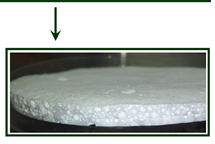
Objective:

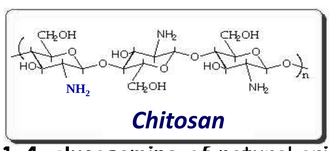
Prepare PDMS based ormosils with biocide activity, by ionizing radiation techniques, to be used alone or as additives in conservation processes of Roman mosaics and/or other stone based ancient structures.

Methods and Results

Preparation: Freeze-dry + γ-Irradiation

Chitosan (2% w/v in CH_3COOH 1%) + **PVA** (5% w/v) + **Gelatin** (2 and 4% w/v)

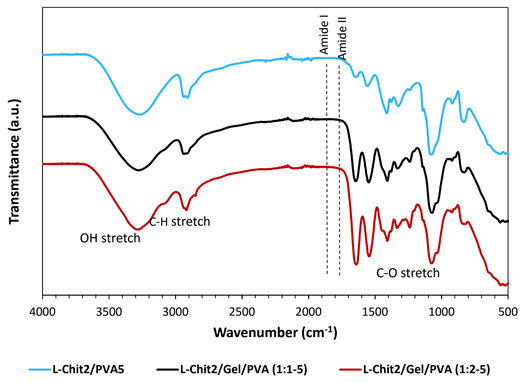
→ **Homogenization + Transference** → ▪ with N_2 bubbling → **Neutralization: C_2H_5OH Washing: H_2O** → **Freeze-drying process** →  → **Sealed in N_2 atmosphere** → **γ Gamma irradiation** *DR=0.5 kGy·h⁻¹ D=10 kGy*



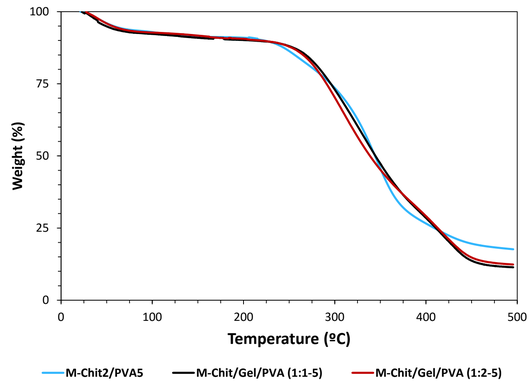
β-1,4-glucosamine of natural origin

Characterization

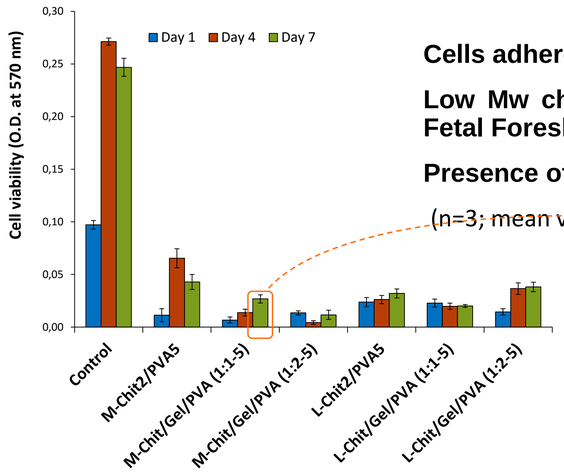
FTIR



TGA



Cellular viability

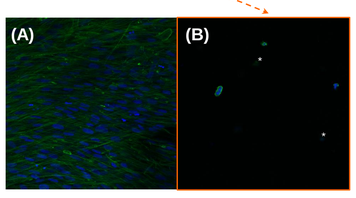


Cells adhered and proliferated in all irradiated matrices

Low Mw chitosan based matrices lead to higher Human Fetal Foreskin Fibroblasts (HFFF2) proliferation

Presence of gelatin improves cells proliferation

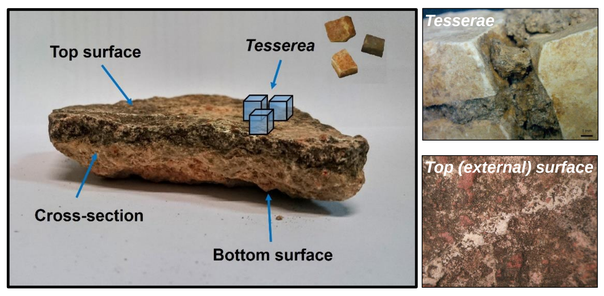
(n=3; mean value ± SD)



(A) HFFF2 control cells (B) HFFF2 cells growing on the in culture for 7 days on M-Chit/Gel/PVA (1:1-5) matrices

Cells display round morphology and poor actin cytoskeletal organization as compared to (A) but are able to invade the depth of the matrices (out of focus cells *; **green actin**; **blue DNA**)

Roman mosaics



PIXE analysis

Surface	Composition (wt%)								
	MgO	Al_2O_3	SiO_2	P_2O_5	$CaCO_3$	TiO_2	MnO	Fe_2O_3	PbO
Top	9,7	11,5	41,4	2,0	29,40	0,5	0,12	3,5	---
Cross-section	6,1	13,0	42,0	0,5	32,10	0,3	0,06	3,3	---
Bottom	4,4	10,1	41,5	1,0	38,40	0,4	0,06	2,2	0,06

Preparation and characterization

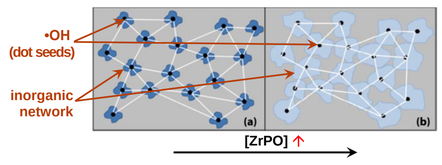
PDMS + TEOS + ZrPO *(in PE or glass sealable containers)* → **Homogenization + Sealing in N_2 atmosphere** → **γ Gamma irradiation** *DR= 10 kGy·h⁻¹* *D ≥ 700 kGy ($D \geq D_{ac}$)*

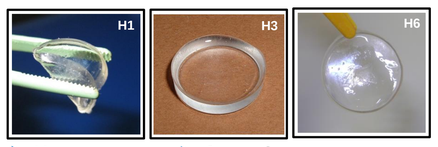
D_{ac} – dose of non-flowing gel

→ **Washing in PDMS solvente** *- Tetrahydrofuran -* → **Solvent evaporation** *(at room temperature)*

Hybrids composition (wt%):

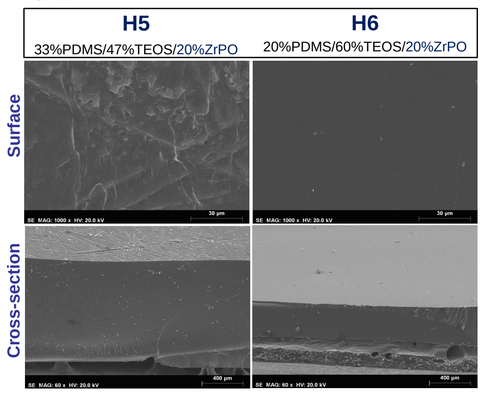
- **H1**: 39%PDMS / 54%TEOS / 7%ZrPO
- **H2**: 33%PDMS / 64%TEOS / 3%ZrPO
- **H3**: 33%PDMS / 62%TEOS / 5%ZrPO
- **H4**: 33%PDMS / 61%TEOS / 6%ZrPO
- **H5**: 33%PDMS / 47%TEOS / 20%ZrPO
- **H6**: 20%PDMS / 60%TEOS / 20%ZrPO





- **Homogeneous**
- **Transparent**
- **Monolithic**
- **Amorphous**
- **↑ [ZrPO] ⇨ ↓ Flexibility**

SEM



- **Homogeneous and compact structure**
- **Relatively smooth surface**
- **No porous structure**

HM's biocide activity

Material	Bacteria			Fungus
	Salmonella 3	*Staphylococcus capitis capitis*	*Non-identified sporulated rods*	*Aspergillus fumigatus*
PDMS	Growth	Growth	Growth	Small growth
H1	Growth	Growth	Growth	Growth
H2	Growth	Growth	Growth	Growth
H3	Growth	Growth	Growth	Growth
H4	Growth	Growth	Growth	Growth
H5	Growth	Small growth	Growth	No growth
H6	Growth	Small growth	Small growth	No growth

Biostatic activity against:

- ***Staphylococcus capitis capitis*** (Gram+)
- **Sporulated rods** (Gram+)
- **Fungi *Aspergillus fumigatus*** (Gram+)

No ionic migration of the active element (Zr) to the surrounding medium (action is localized) <=> Good structural stability of *HM's*

Conclusions

- **Typical peaks related to Chitosan, Gelatin and PVA are present;**
- **The introduction of Gelatin into Chitosan based matrices does not change significantly its structural stability;**
- **The evaluation of cell viabilty showed that HFFF2 cells adhered to the surface of all matrices, that did not present cytotoxicity, but proliferated less than in control;**
- **Low Mw Chitosan and introduction of Gelatin revealed to be favorable to cellular growth;**
- **Results show good perspectives for the intended use.**
- **All HM's prepared showed to be stable and with a good homogeneity;**
- **Even HM's with the highest [ZrPO] and lower [PDMS] showed to be stable keeping a characteristic nanostructure;**
- **HM's biostatic activity is a function of [ZrPO];**
- **Biocide assays did not evidence the ionic migration of Zr to the surrounding medium (preventing possible future environmental contaminations);**
- **Biocide/biostatic activity of HM's must be improved and enlarged for a group of other potencial danger microorganisms (algae and cyanobacteria).**

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