Department of Biomedical Sciences-IN2UB Meeting

July 9th 2019, Faculty of Medicine (Campus Clinic)

Stimulus Triggered Delivery Systems for Chronic Wound Healing

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Cellular Responses to Xenobiotics (CEREX)

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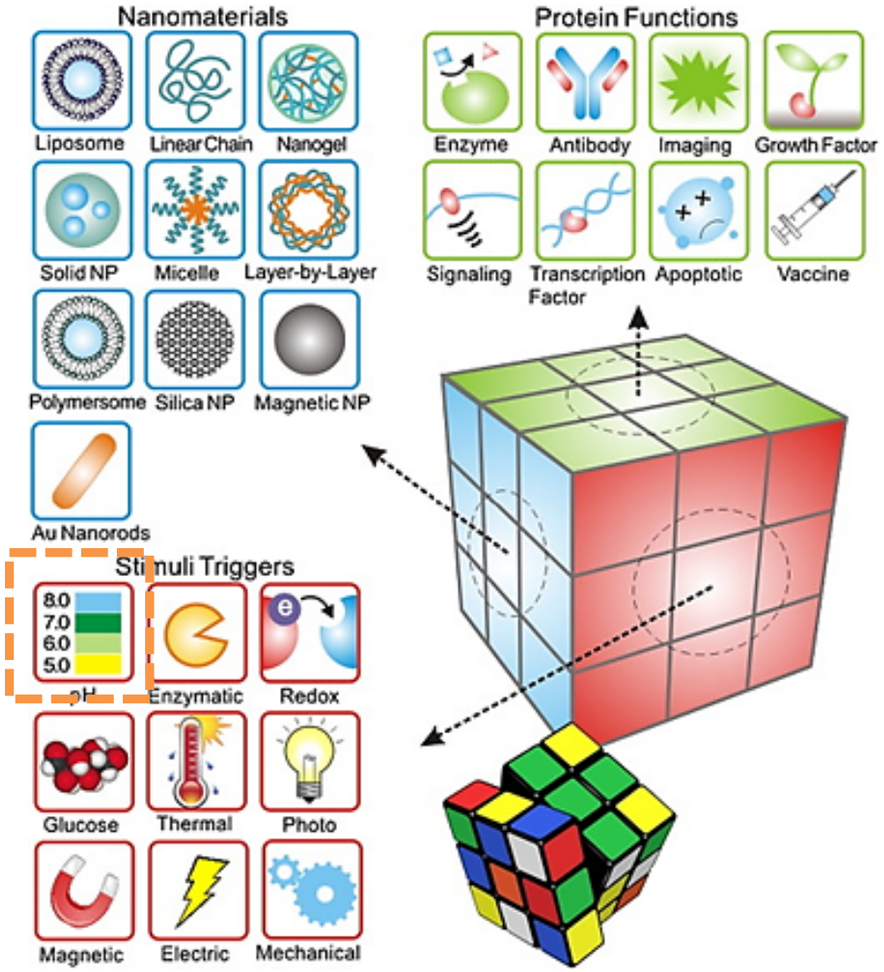
Facultat de Farmàcia i Ciències de l'Alimentació

Departament de Bioquímica i Fisiologia

Therapeutic Applications of Stimulus Triggered Delivery Systems



Orders of Active Targeting

Intercellular organelles targeting

Drug delivery specifically to the intracellular organelles of the target cells

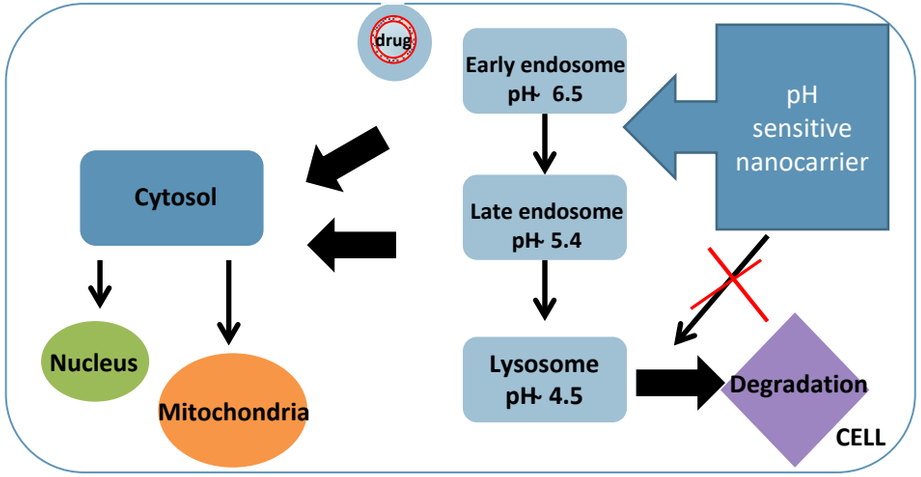
Cellular targeting

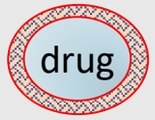
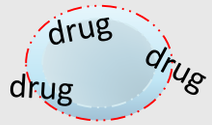
Selective drug delivery specifically to cell type (such as tumor cells and not to the normal cells)

Organ compartmentation targeting

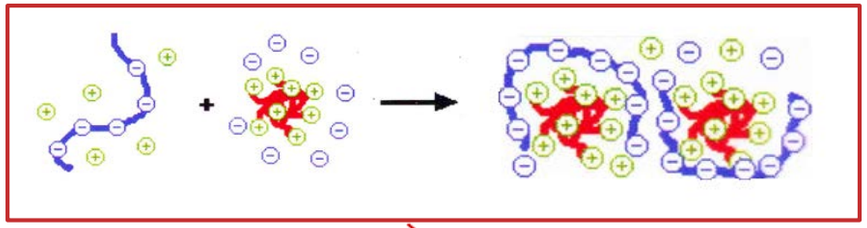
Restricted distribution of the drug system to a pre-determined organ

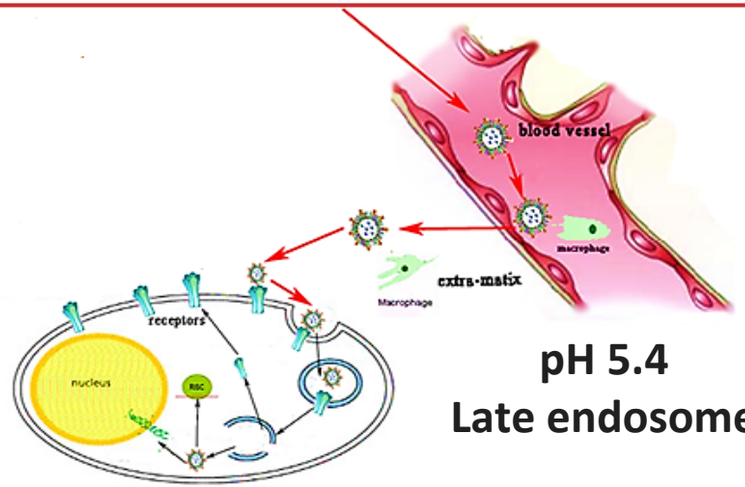
i. Effective intracellular delivery of nucleic acids



	pH 7.4	pH 5.4
Gelatin type B pI 4.8-5.5	-	+
PS pI 12	+	+
Expected vehicle		

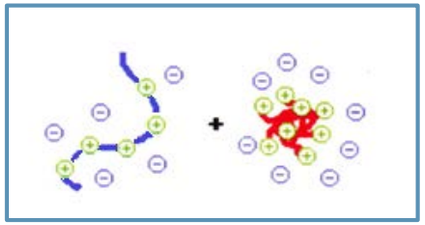
pH 7.4 In circulation



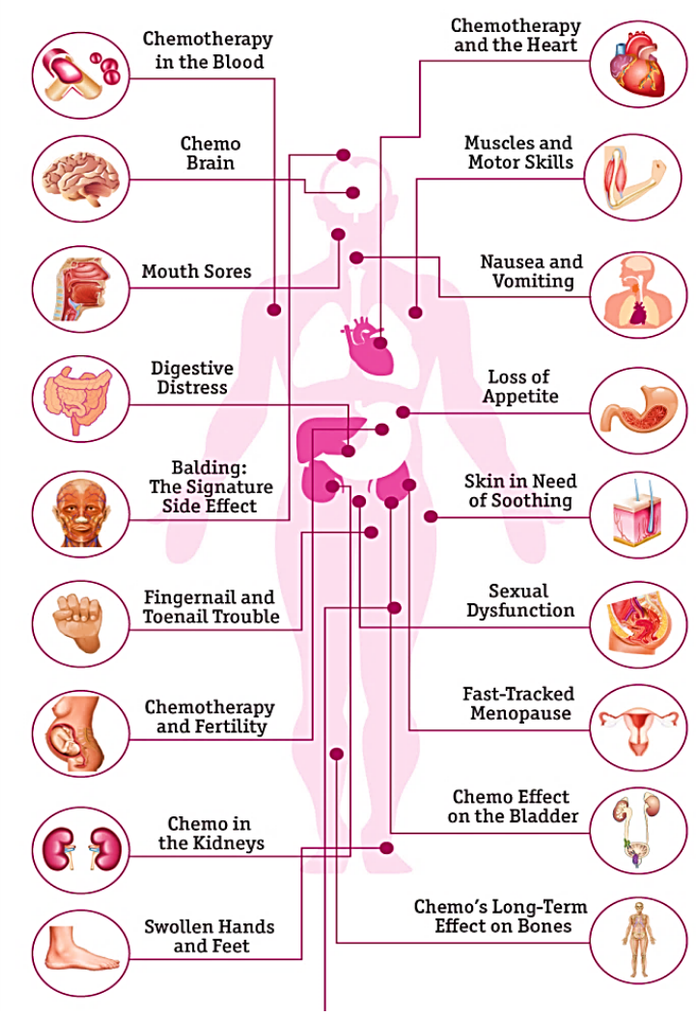


pH 5.4
Late endosome

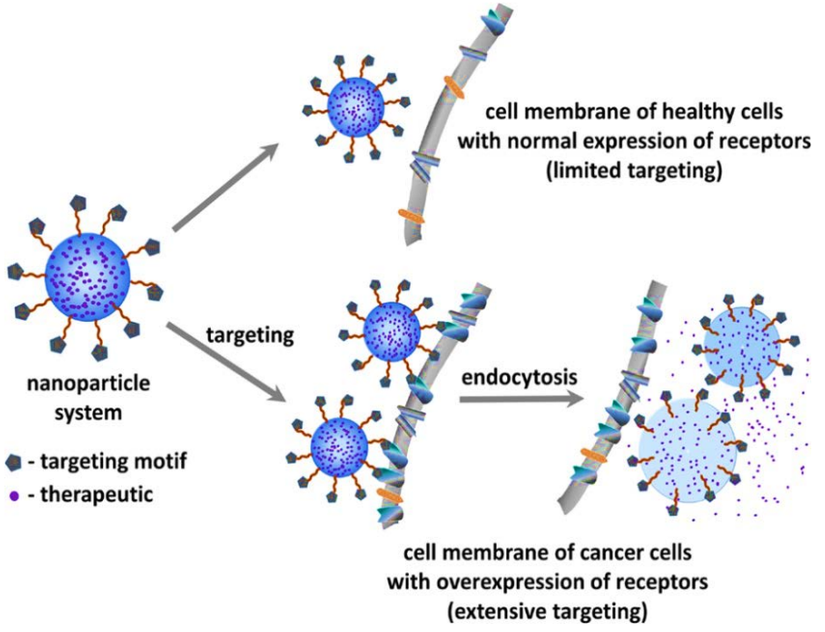
charge inversion



ii. Selective toxicity on chemotherapy



Integrin-mediated endocytosis



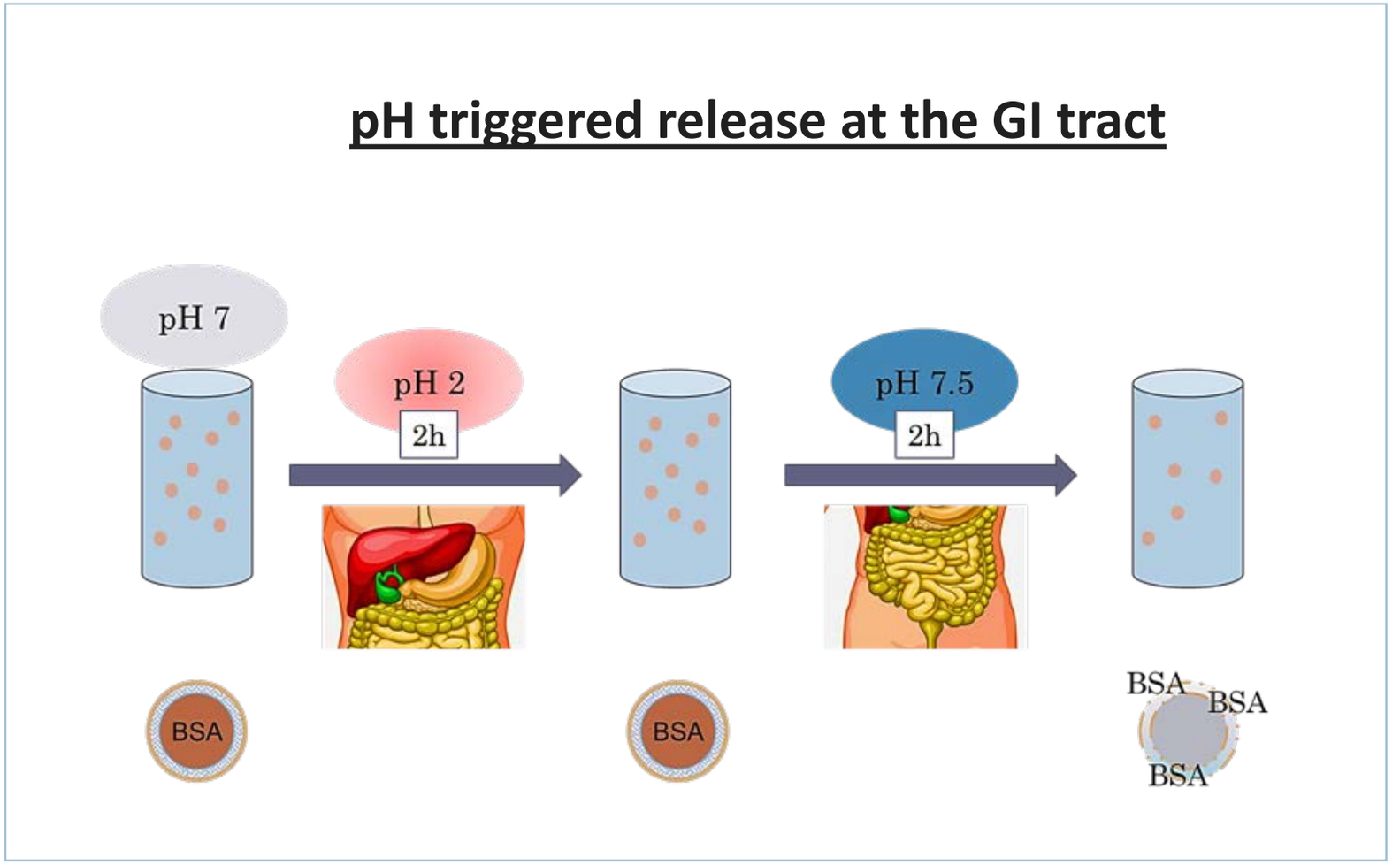
TARGETING MOTIF

Arginine-glycine-aspartic acid (RGD)-peptide present at the gelatin sequence

iii. Challenges for the oral delivery of proteins

Why:

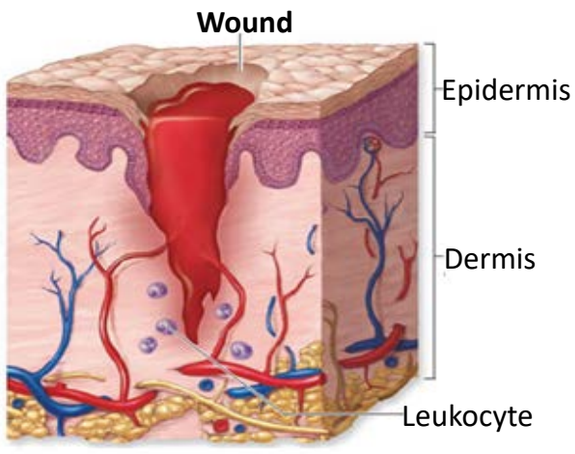
- Increase patient compliance and comfort
- Mimic physiological delivery of proteins
- Simple administration
- Reduce costs
- Potentially improve efficacy



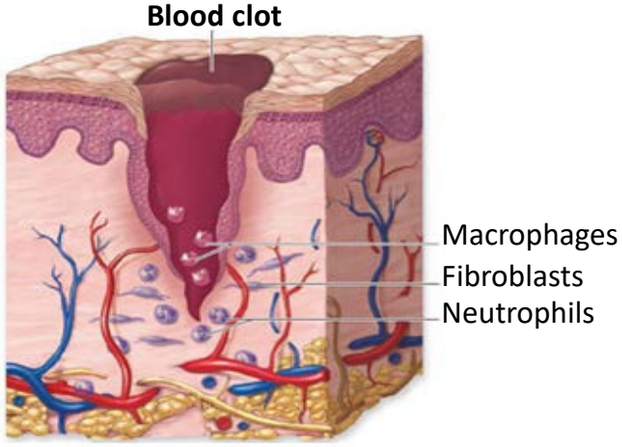
Skin Wound Healing

Deep Wound Healing

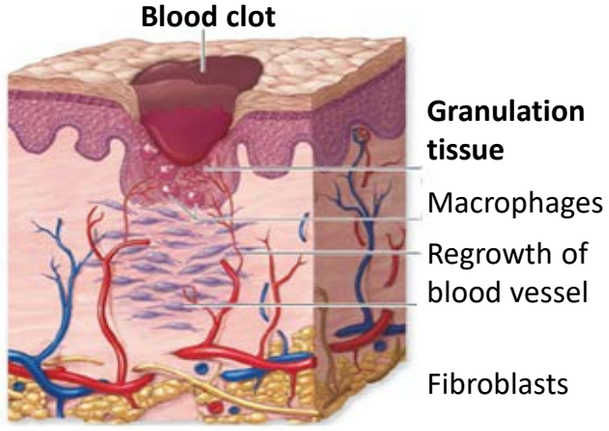
Deep wound healing occurs when an injury extends to the **dermis and subcutaneous layer**. Because multiple tissue layers must be repaired, the healing process is more complex than in epidermal wound healing. In addition, because scar tissue is formed, the **healed tissue loses some of its normal function**.



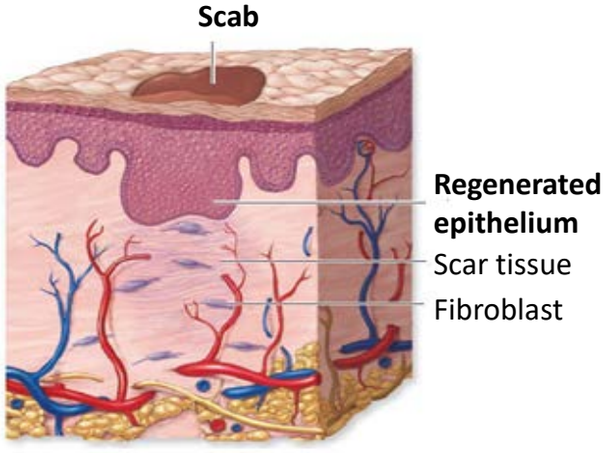
Hemostasis
(Duration = Minutes)



Inflammation
(Duration = Days)



Proliferation
(Duration = Weeks)



Remodeling
(Duration= 1 Year +)

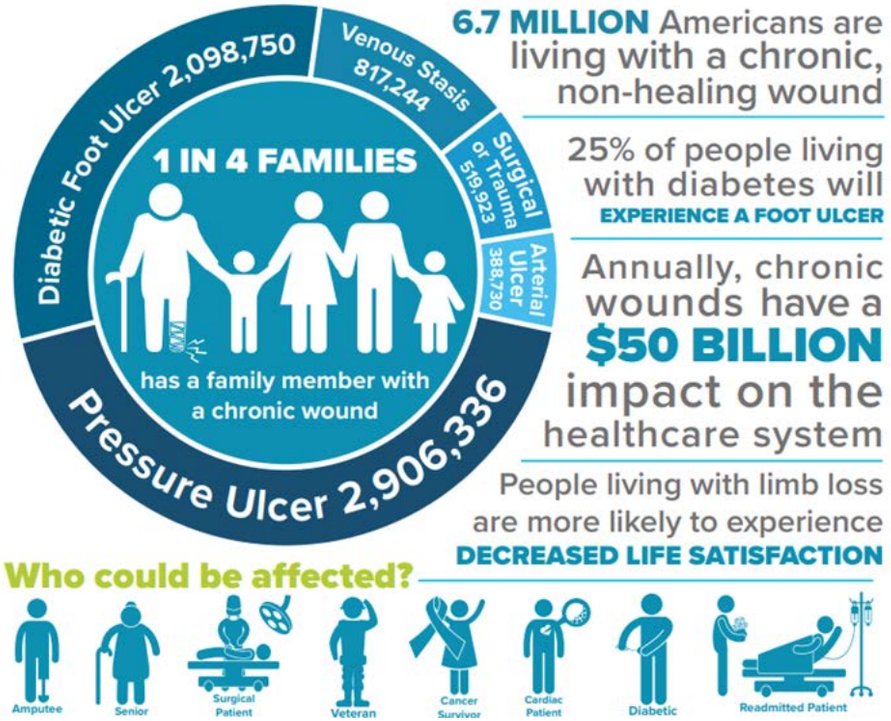
Chronic Wounds are a Major Public Health Concern

Chronic wounds do not follow normal healing processes and timelines. They seem to be detained in one or more of the phases of wound healing (often **remain in the inflammatory stage** for too long).

Wounds that do not heal within **three months** are often **considered chronic**.

Chronic diseases affecting Wound Healing

- Arterial insufficiency
- Surgical/Trauma Wounds
- Venous insufficiency
- Diabetic Foot Ulcer
- Pressure Ulcer
- Defective extracellular matrix (ECM) turnover

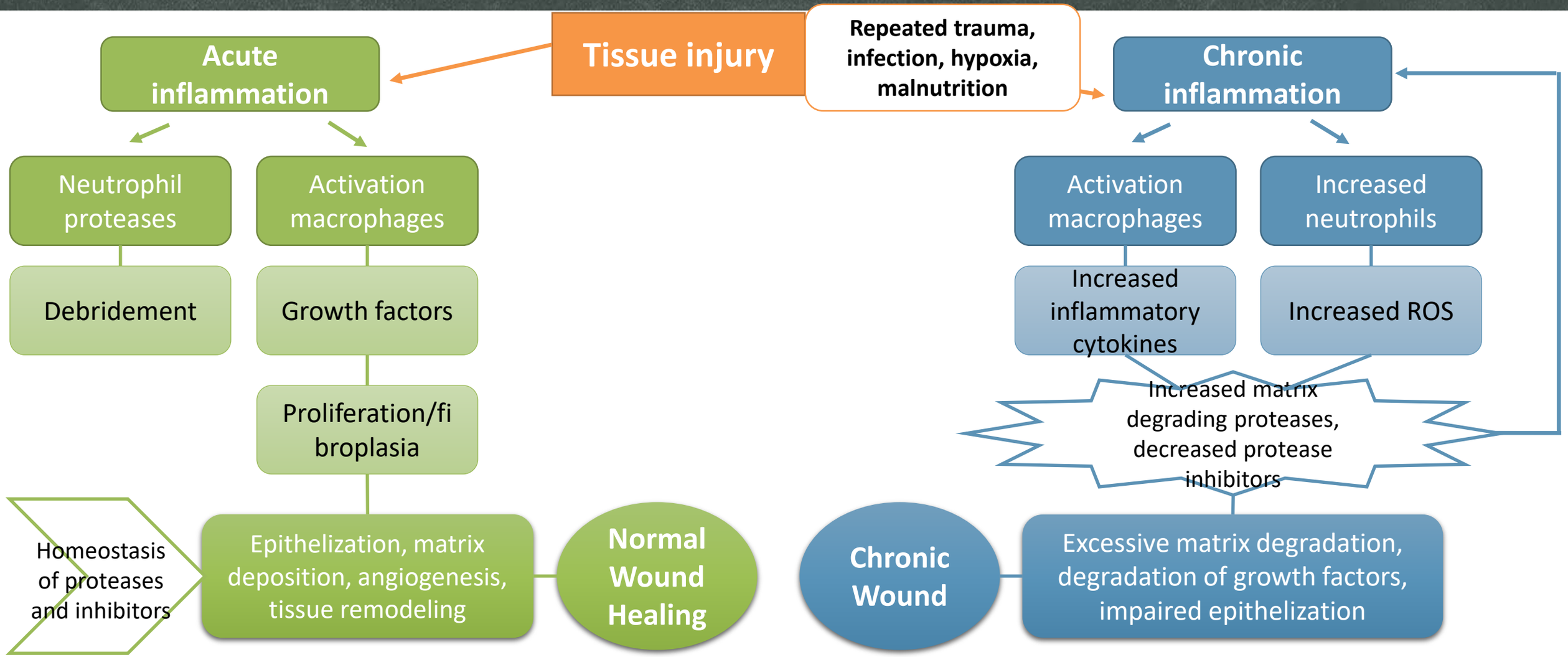


Estimation: **1-2% total population** will experience a **chronic wound during their lifetime**

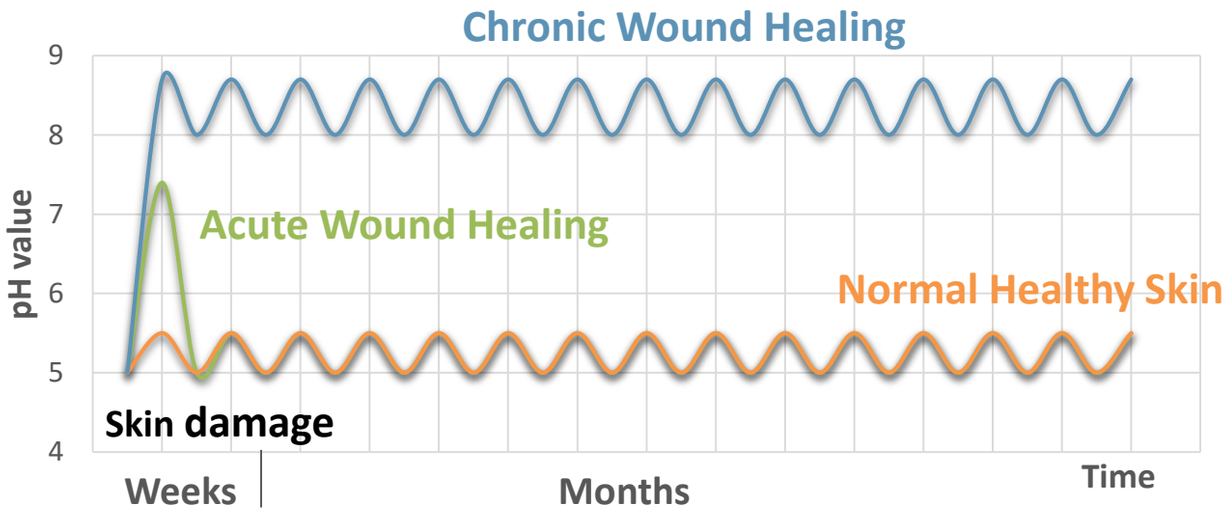
The treatment of chronic wound affects public healthcare system spending **5 % of total health spending in Spain**

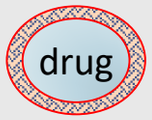
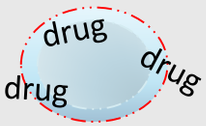
The **incidence became trebled for older people** (> 75 years)

Pathway for Acute and Chronic Wounds



Effect of pH on Wound Healing



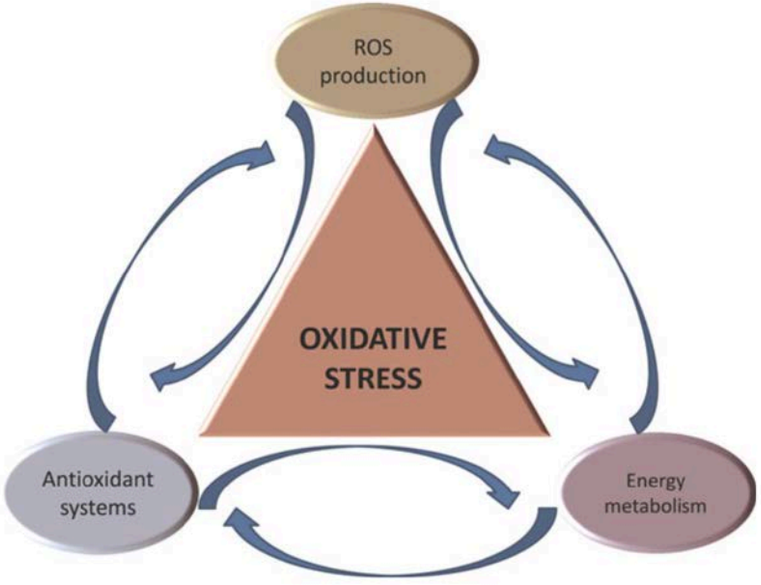
	pH 5.5	pH 7.15-8.93
Gelatin type B pI 4.8-5.2	-	-
Gelatin type A pI 7 (low bloom) pI 9 (high bloom)	+	-
Expected vehicle		

- **Healthy skin** has a slightly **acidic pH ranging from 4.0 to 6.0**
- **After a wound**, skin exposes a **more neutral pH (7.4)**
- **With successful healing** , the skin **returns to being ac**idic
- **When chronicity** occurs, the **pH become increasingly alkaline over time**
- **Chronic wound environmen**t have a **pH ranging from 7.15 to 8.93**

Gelatin-based NPs for Wound Healing

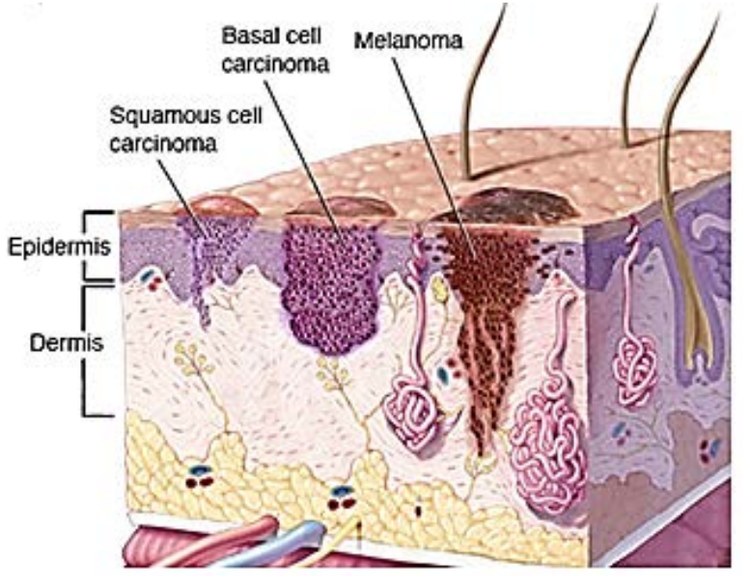
Oxidative stress

- Antioxidants (Ascorbic acid)

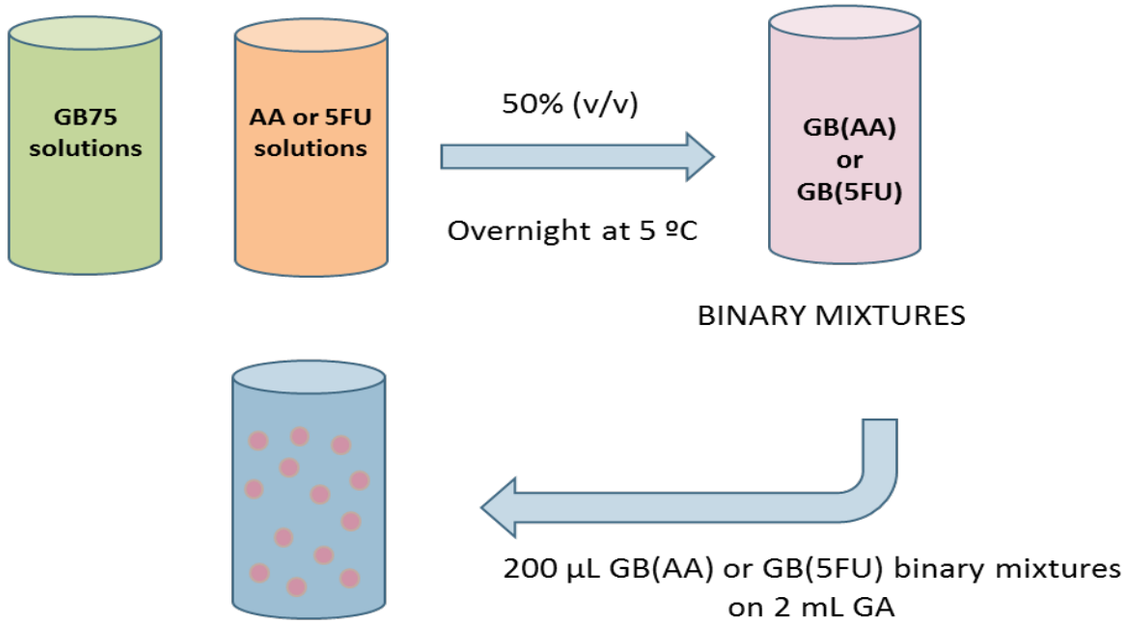


Malignancy on Chronic Wounds

- Antitumoral drug (5-FU)



Physicochemical characterization of gelatin-based NPs



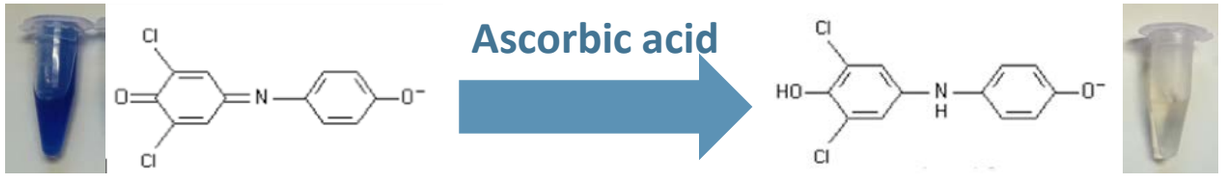
GB(AA)-GA NPs / GB(5FU)-GA NPs

NanoPhotometerTM

Zetasizer Nano ZS90

Parameters	GB(AA)-GA NPs	GB(AA)-GA NPs
Size	200-700nm	60-130 nm
pdI	0.4-0.7	0.5-0.7
LE	10-80%	30-60%

2,6-Dichlorophenol indophenol (DCPIP)

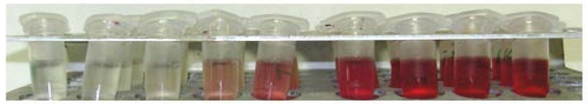


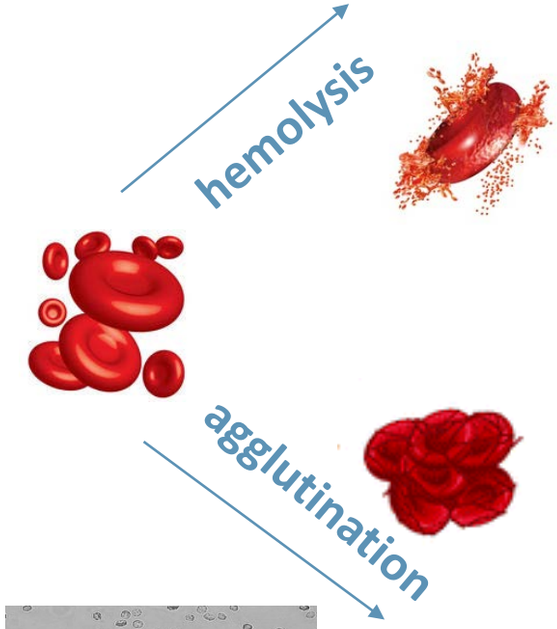
5-FU λ=267 nm

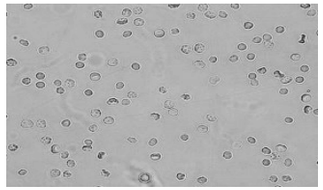
$$LE(\%) = \frac{[Drug]total - [Drug]free}{[Drug]total} x100$$

Hemolysis and agglutination as expression of NPs-induced cytotoxicity in human red blood cells

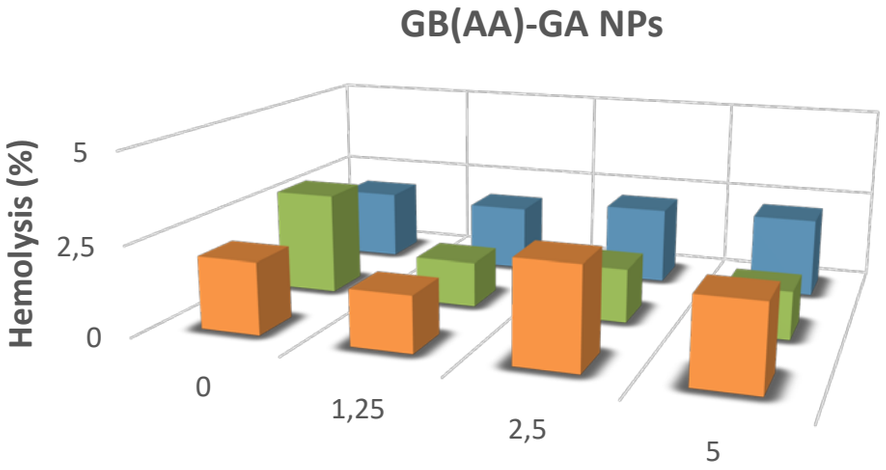
0% ▸ 20% ▸ 50% ▸ 100%

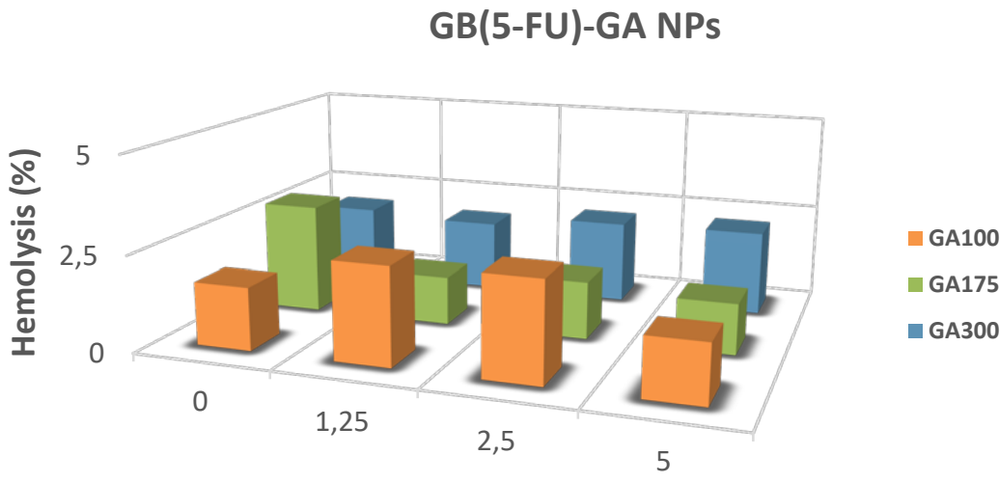




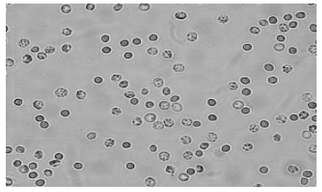


RBC





NPs are not hemolytic (< 5% that is stipulated by the ISO 10993-4)



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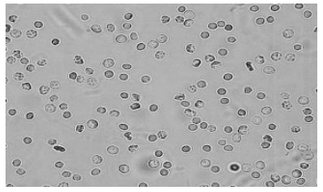
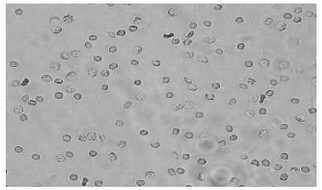
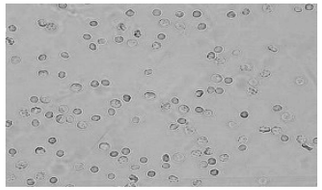


1.25



2.5



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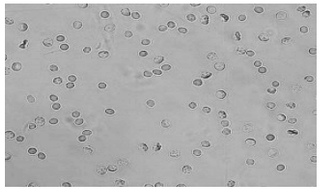

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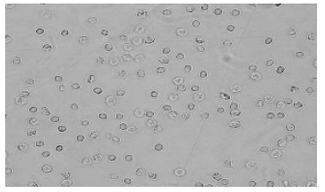

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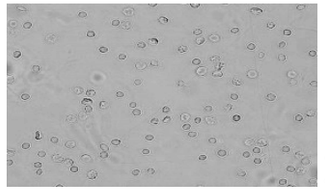

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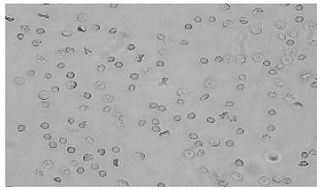
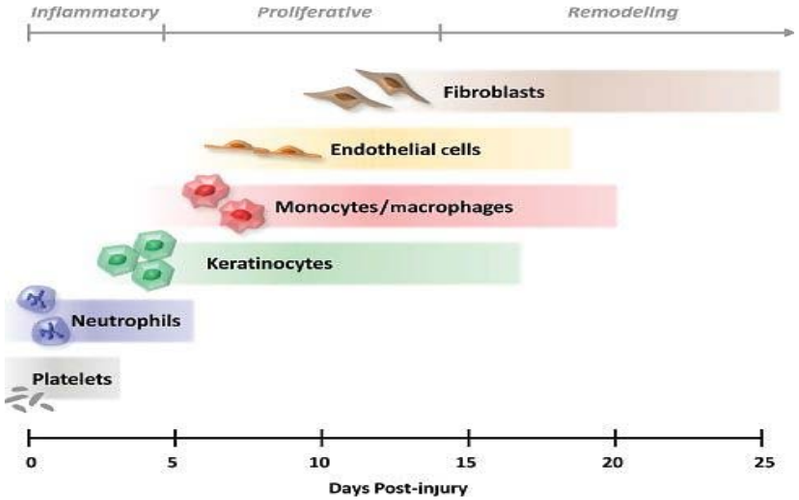


5

Interaction with cells

During healing, the new tissue formation is characterized by two major processes:

1. **Re-epithelization** in which the **migration and proliferation of keratinocytes** leads to the first epidermal wound closure
2. **The granulation tissue formation** in which **components of the new extracellular matrix** such as collagen , elastic structures, glycoproteins and proteoglycans **are released by the invaded fibroblasts**

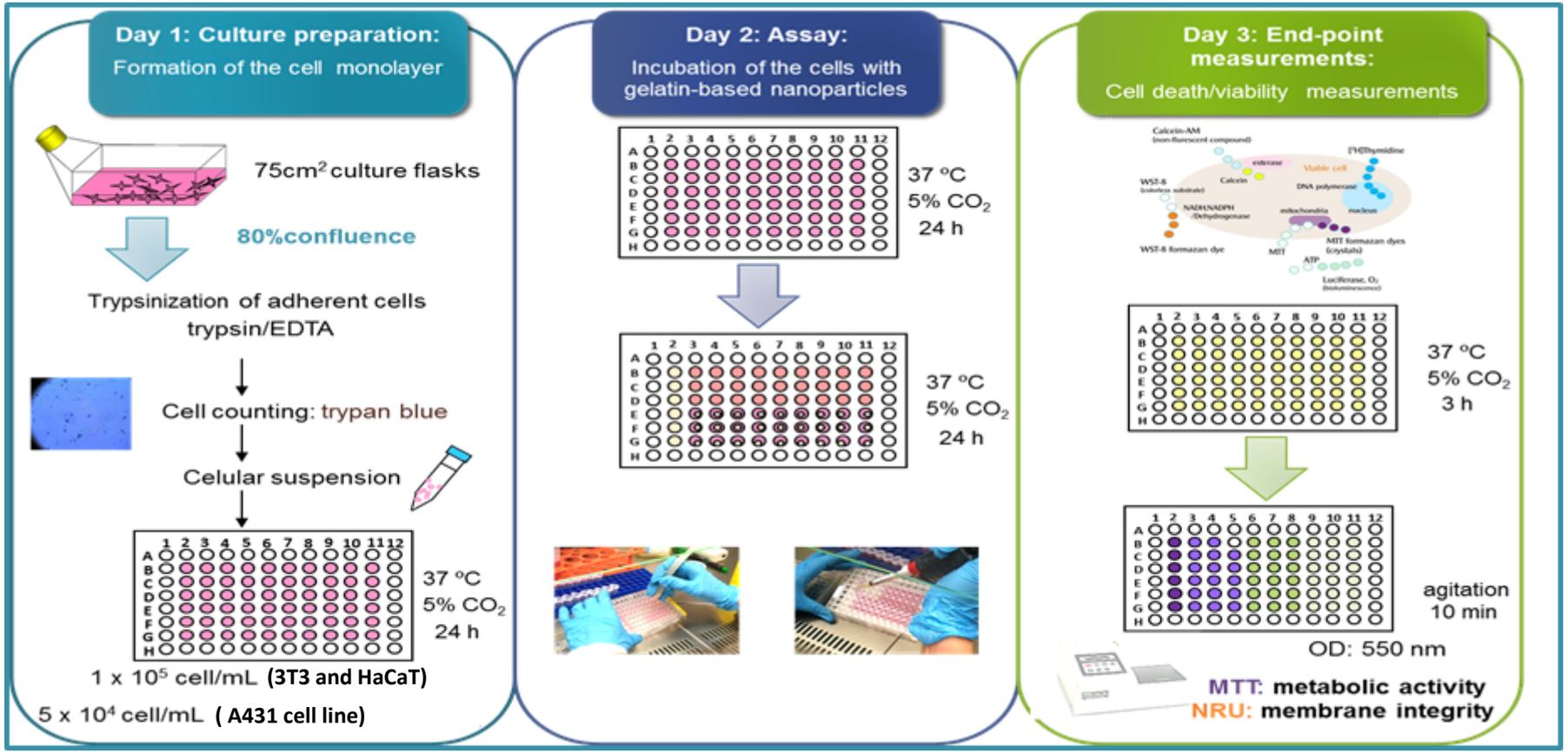


Fibroblasts and keratinocytes cells

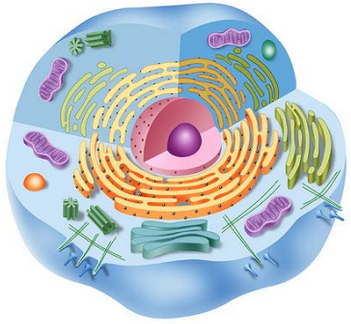
- Fibroblasts (3T3 cell line)
- Keratinocytes (HaCaT and A431 cell lines)

- **Cytotoxicity and proliferation:**
  - MTT assay and vital stainings
- **Antioxidant properties**:
  - Hydrogen peroxide as induced oxidative stress

In vitro cell viability



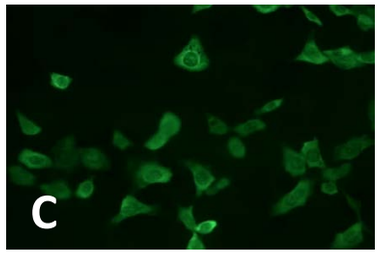
5.5 7.4 9.0

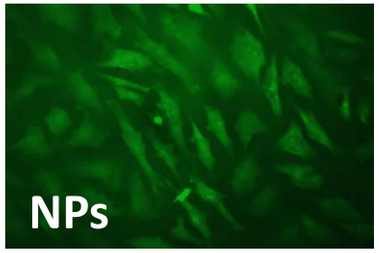


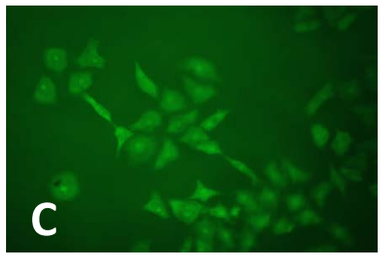
MTT

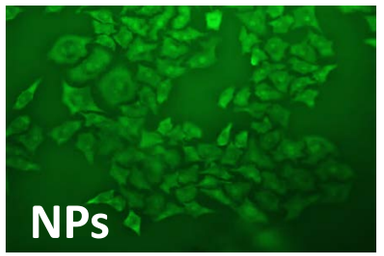
Mitochondrial metabolism

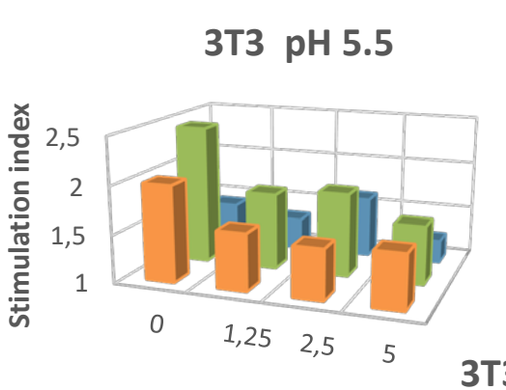
Proliferative effect on cells

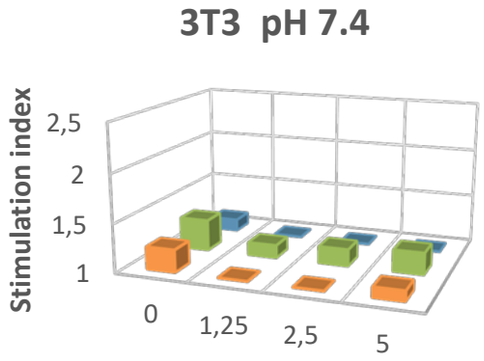


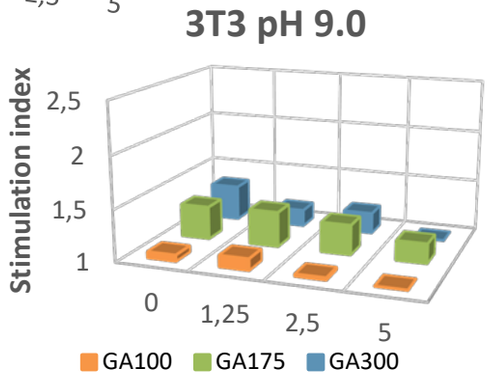




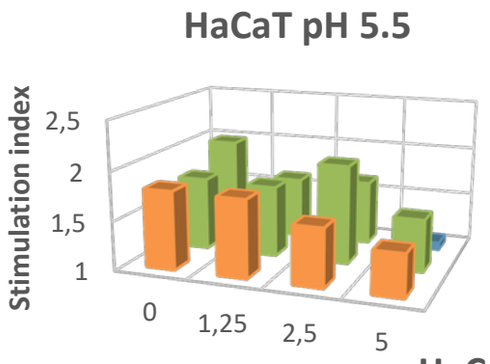


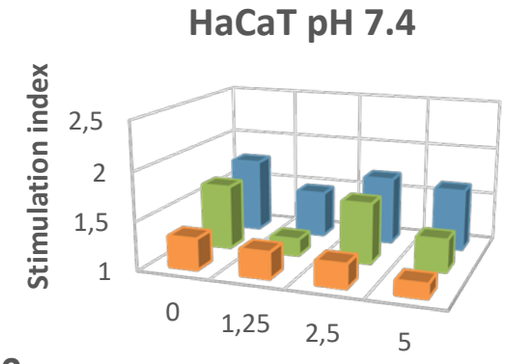


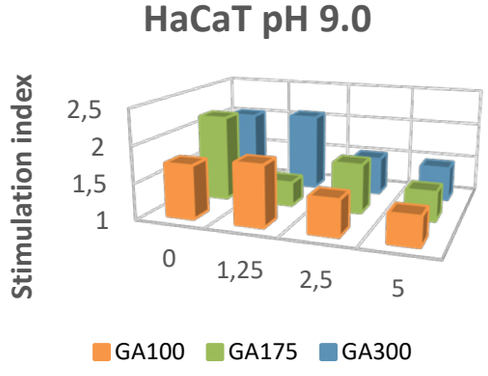




3T3
SI: **1-2.5**

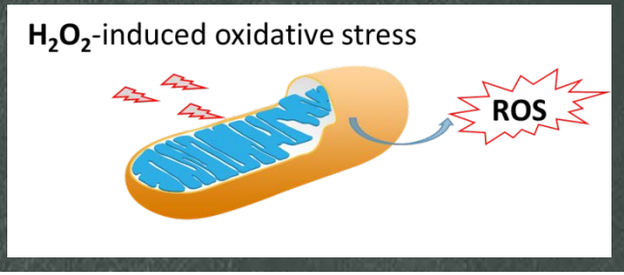


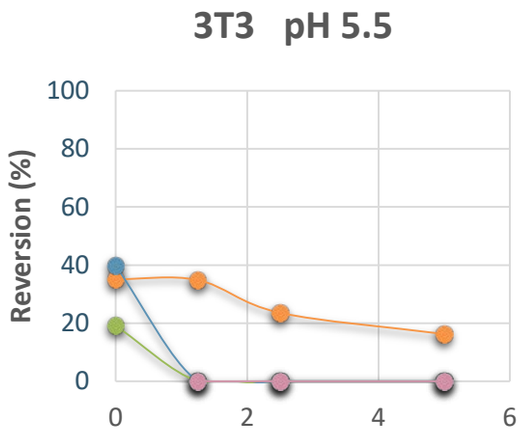


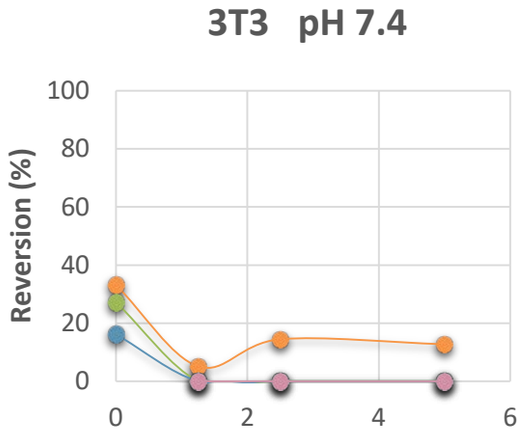


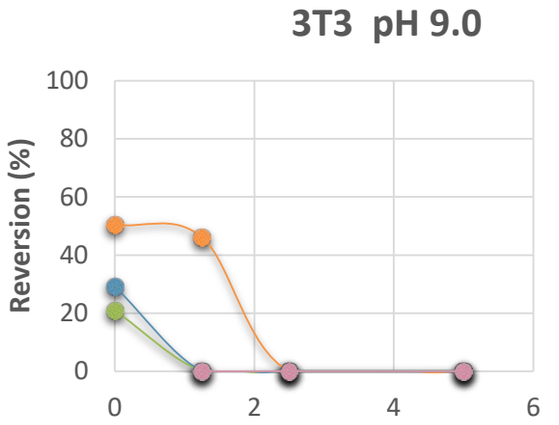
HaCaT
SI: **1.5-2**

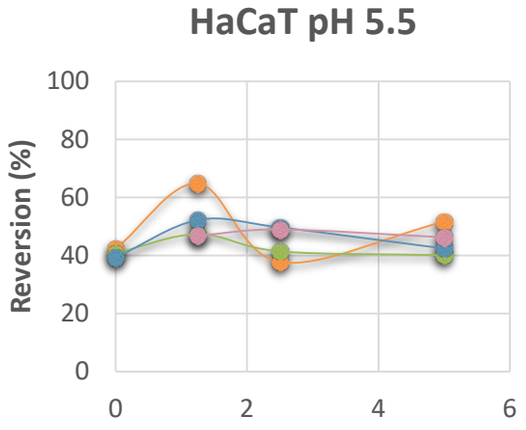
Antioxidant properties of AA-containing NPs

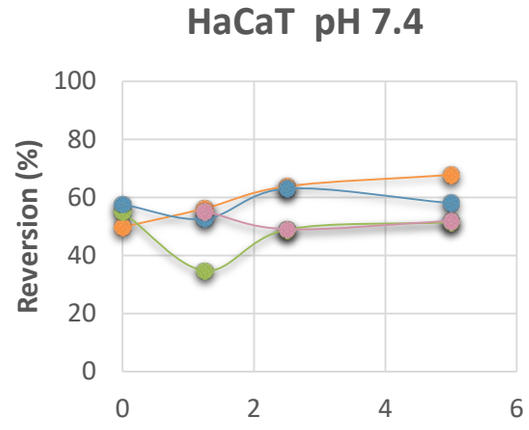


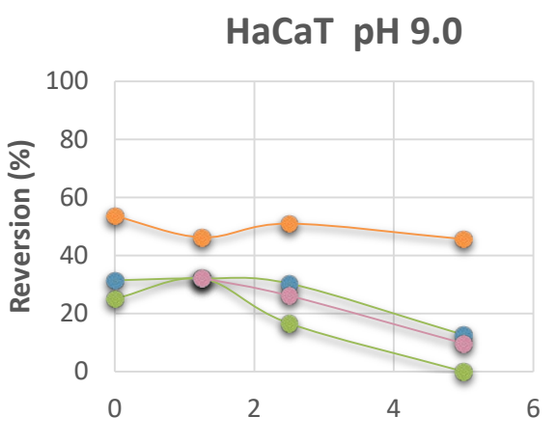












3T3

H_2O_2: 52.6%

10-30%

HaCaT

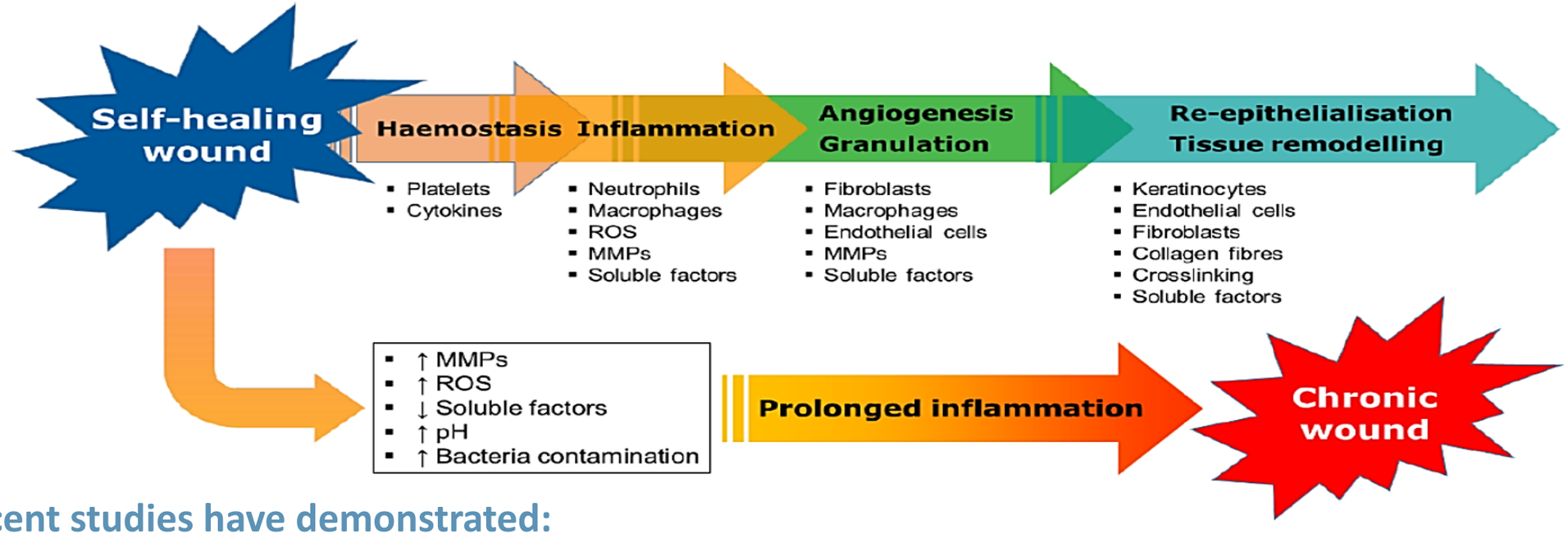
H_2O_2: 33.4%

40-60%

Selective cytotoxicity induced by 5FU-containing NPs

		Euglycemic conditions		Hyperglycemic conditions	
	System	SI (3T3/A431)	SI (HaCaT/A431)	SI (3T3/A431)	SI (HaCaT/A431)
pH 5.5	GA100 NPs	>5.20	1.06	1.05	>3.90
	GA175 NPs	>4.95	0.91	0.81	>3.05
	GA300 NPs	>4.03	1.09	1.04	>3.79
pH 7.4	GA100 NPs	>7.46	1.49	2.10	1.97
	GA175 NPs	>4.80	>4.80	0.88	>3.60
	GA300 NPs	>2.11	>2.11	0.81	>3.97
pH 9.0	GA100 NPs	>5.81	1.19	1.47	1.26
	GA175 NPs	>4.81	>4.81	0.90	>3.47
	GA300 NPs	>3.76	>3.76	1.15	>4.90

Concluding remarks



Our recent studies have demonstrated:

- **The capability of building NPs with simulated chronic wound pH-triggered release properties.**
- **Biocompatible NPs with non hemolytic and non cytotoxic properties.**
- **Enhanced *in vitro* fibroblasts and keratinocytes proliferation mediated by NPs.**
- **Protection against hydrogen peroxide-induced oxidative stress mediated by AA-containing NPs.**
- **Selective cytotoxicity induced by 5-FU containing NPs.**

Collaborations

COST Action

CM1101-Polymer & surfactant self-assembly: Formulations, deposition and nanoparticle formation.

MP1106- Smart and green interfaces - from single bubbles and drops to industrial, environmental and biomedical applications.

Codi GREC 18407- Framework Cooperation Agreement Faculty of Pharmacy and Food Science **(UB)-** Institute for Chemistry of Condensed Matter and Technologies for Energy (**ICMATE-CNR)**

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CEREX

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Dr. Montse Mitjans
Cristina Porredón
Coloma Gibert
Albert Ferriol

COLOIDS

Physicochemical characterization

NPs preparation and biological characterization

Surface functionalization and cell behaviour