



Hematology Journal Club

Dose-Dependent Effects of Platelet-Rich Plasma Powder on Chondrocytes In Vitro

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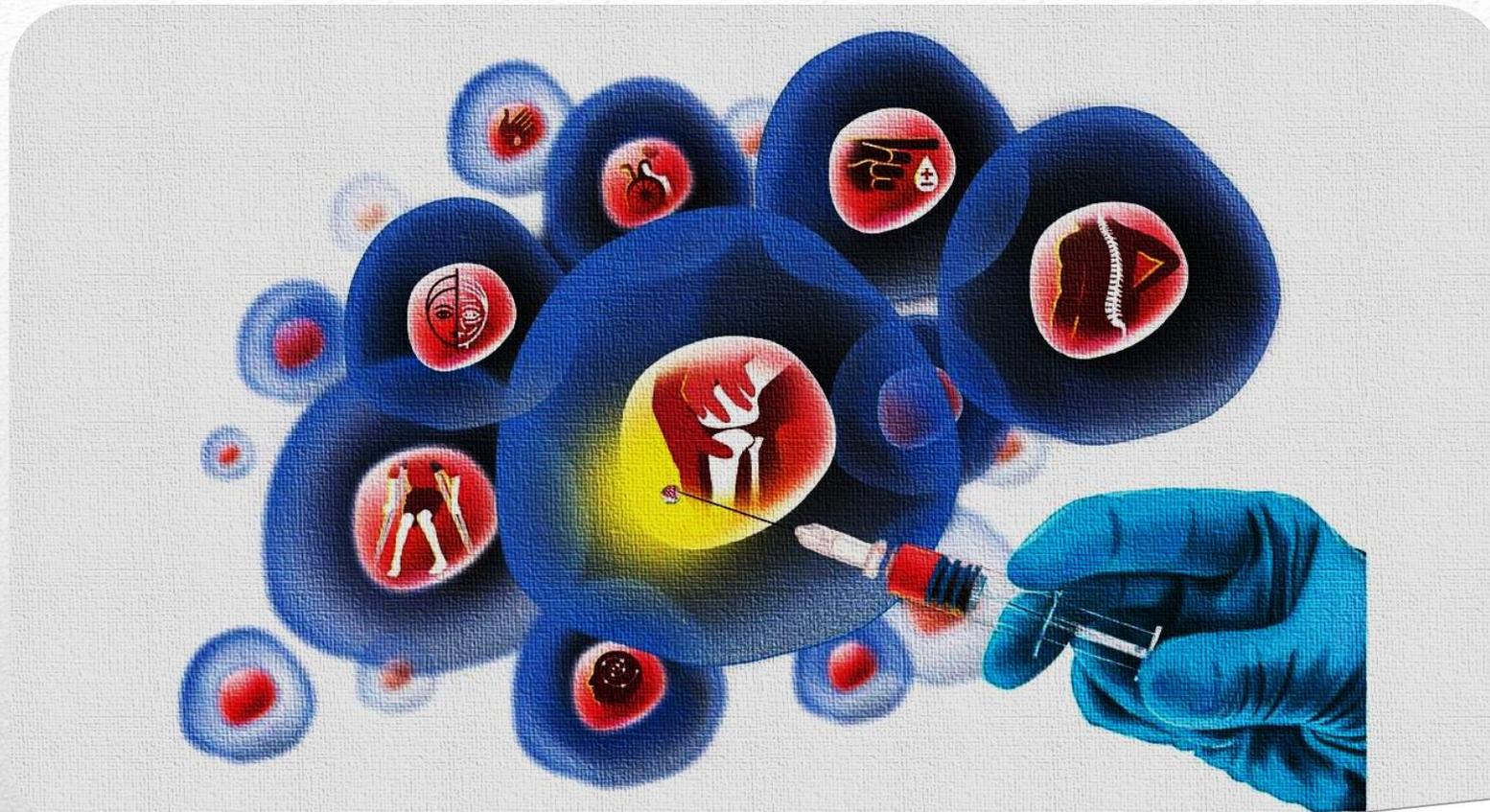
Aims and Scope

The American Journal of Sports Medicine, founded in 1972, is the official publication of the American Orthopaedic Society for Sports Medicine. It contains original articles addressed to orthopaedic surgeons specializing in sports medicine, and to team physicians, athletic trainers, and physical therapists focusing on the causes and effects of injury or disease resulting from or affected by athletic injury.



The American journal of Sports Medicine

Cell Therapy



Regenerative
Medicine

Orthopedic surgery & Sports Medicine

- Tendinosis, Tendinopathy
- Acute and chronic muscle strain
- Ligamentous sprain
- Joint capsular laxity
- Arthritis



Maxillofacial, Cosmetic & Plastic surgery

- Anti-aging treatment
- Acne marks treatment
- Facial Laser treatment
- Fat transfers
- Hair loss treatment



PRP

Podiatric surgery

- Diabetic foot ulcers



Dental surgery

- Implant



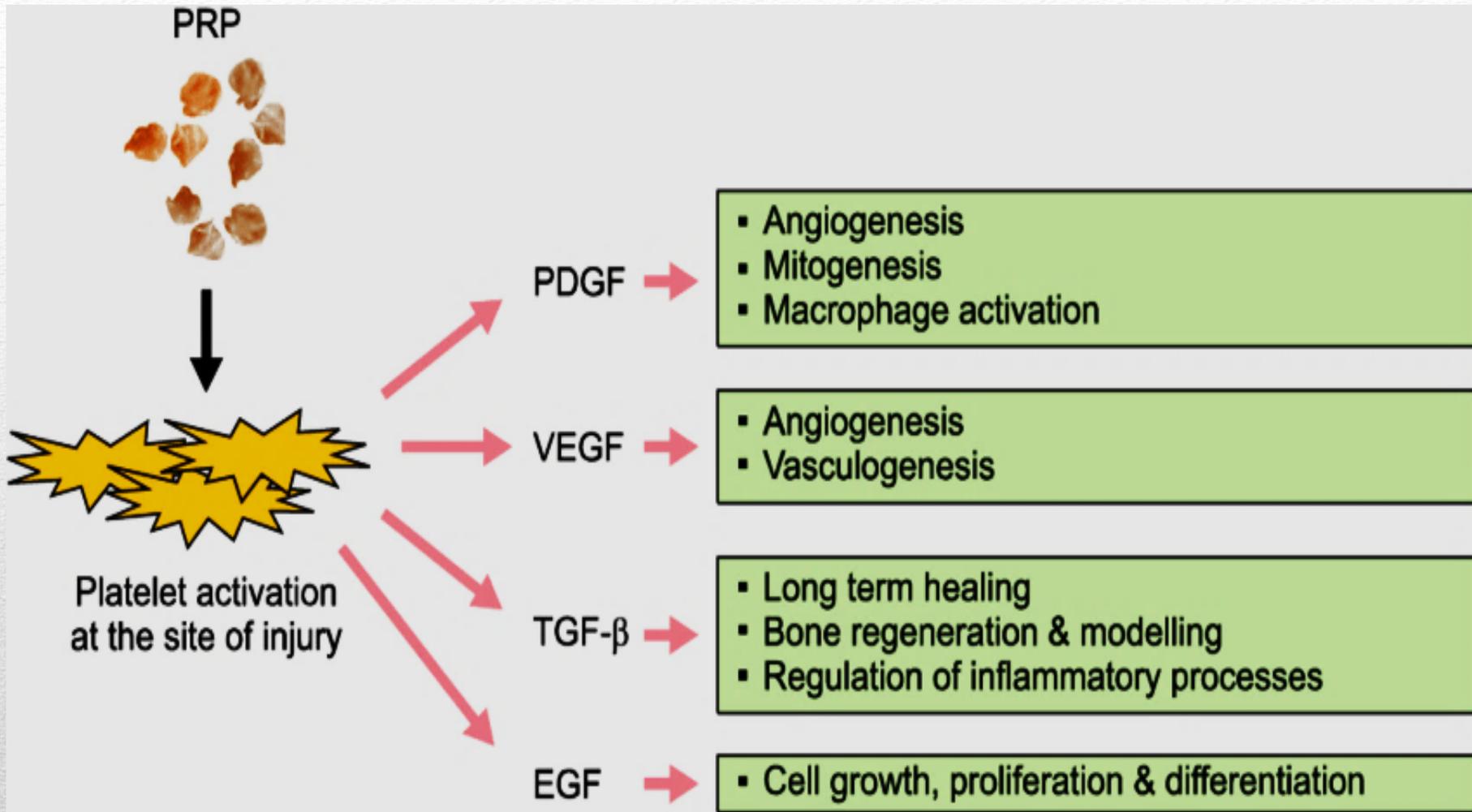
Ophthalmic surgery

- Refractive surgery (LASIK, LASEK)



PRP Applications





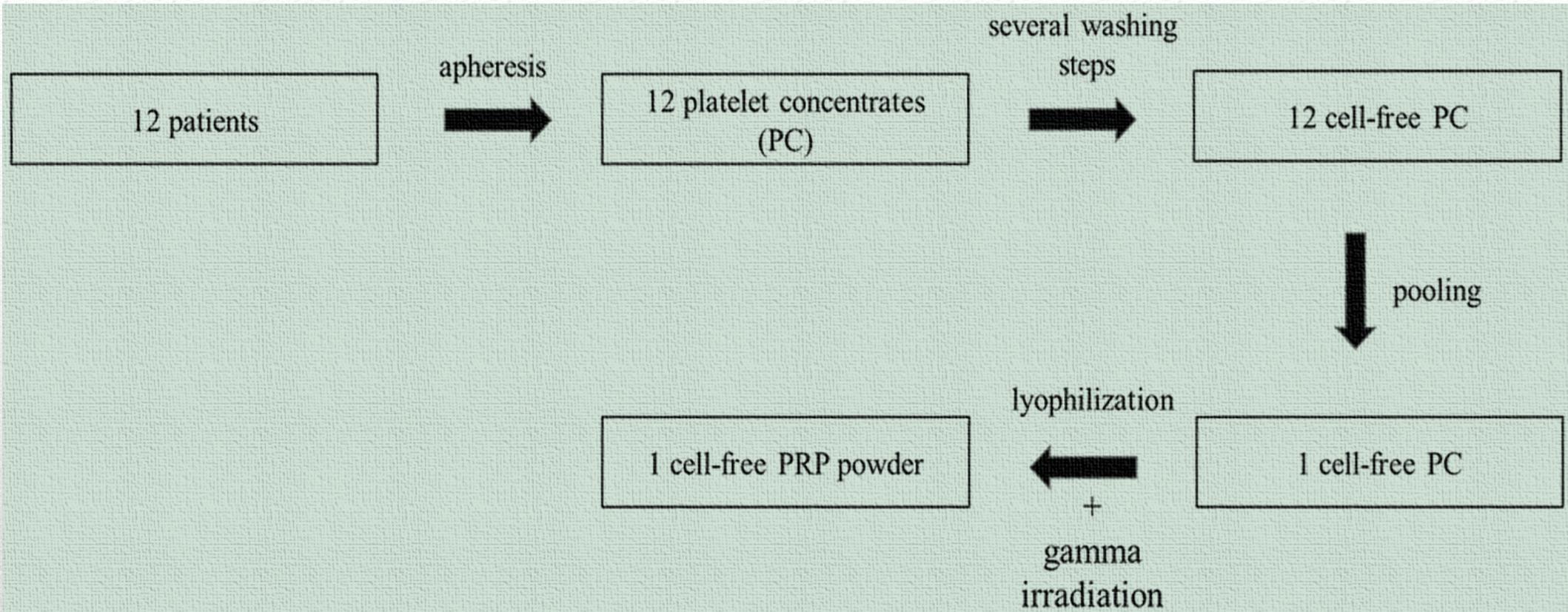
Uncertainty of the composition of the main components

**non-standard production processes and variability
in the properties of the final platelet products**

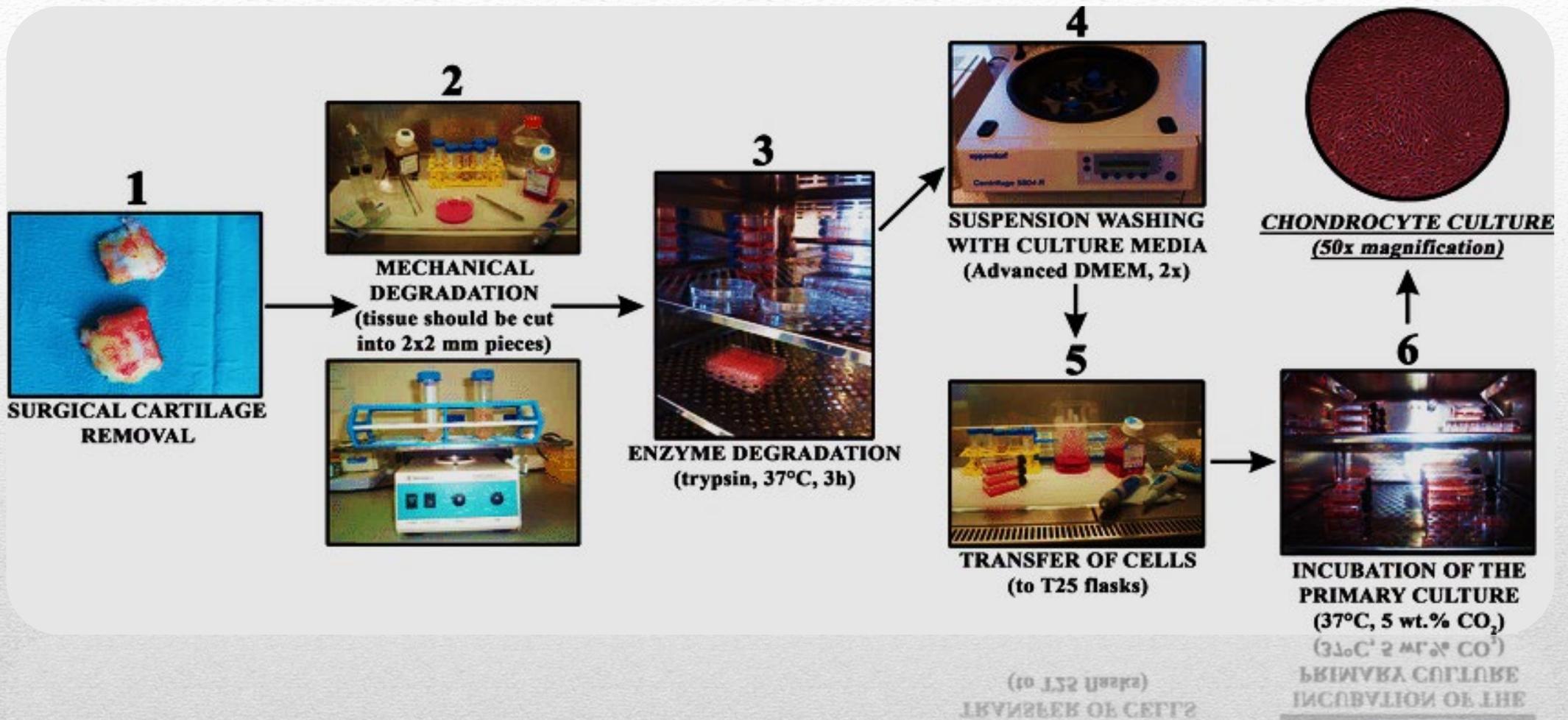
Uncertainty of the concentration of growth factors

Limited shelf life

need to the development of a standardized PRP powder with characterized growth factors, which would allow reproducible testing of the effects of different parameters, such as PRP concentration, amount of PRP injections, and timing of injections in vitro and in vivo



METHODS:1. Standardized Lyophilized Platelet Growth Factor Preparation

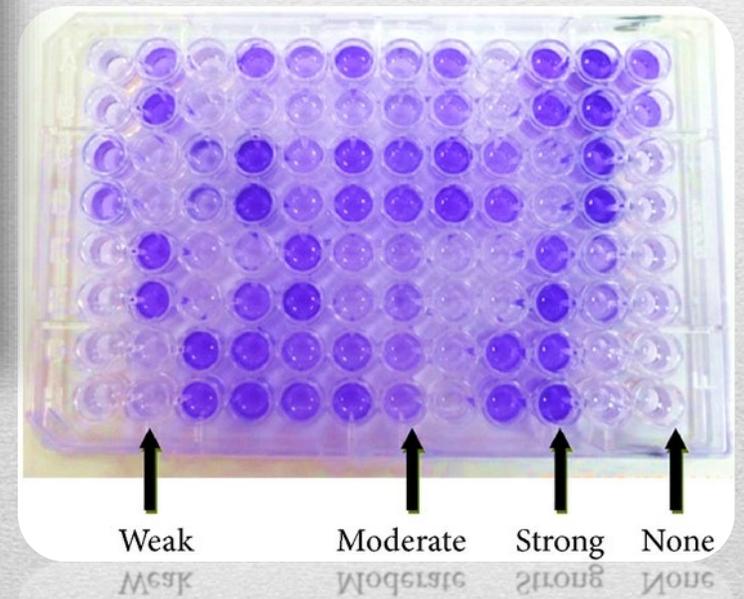
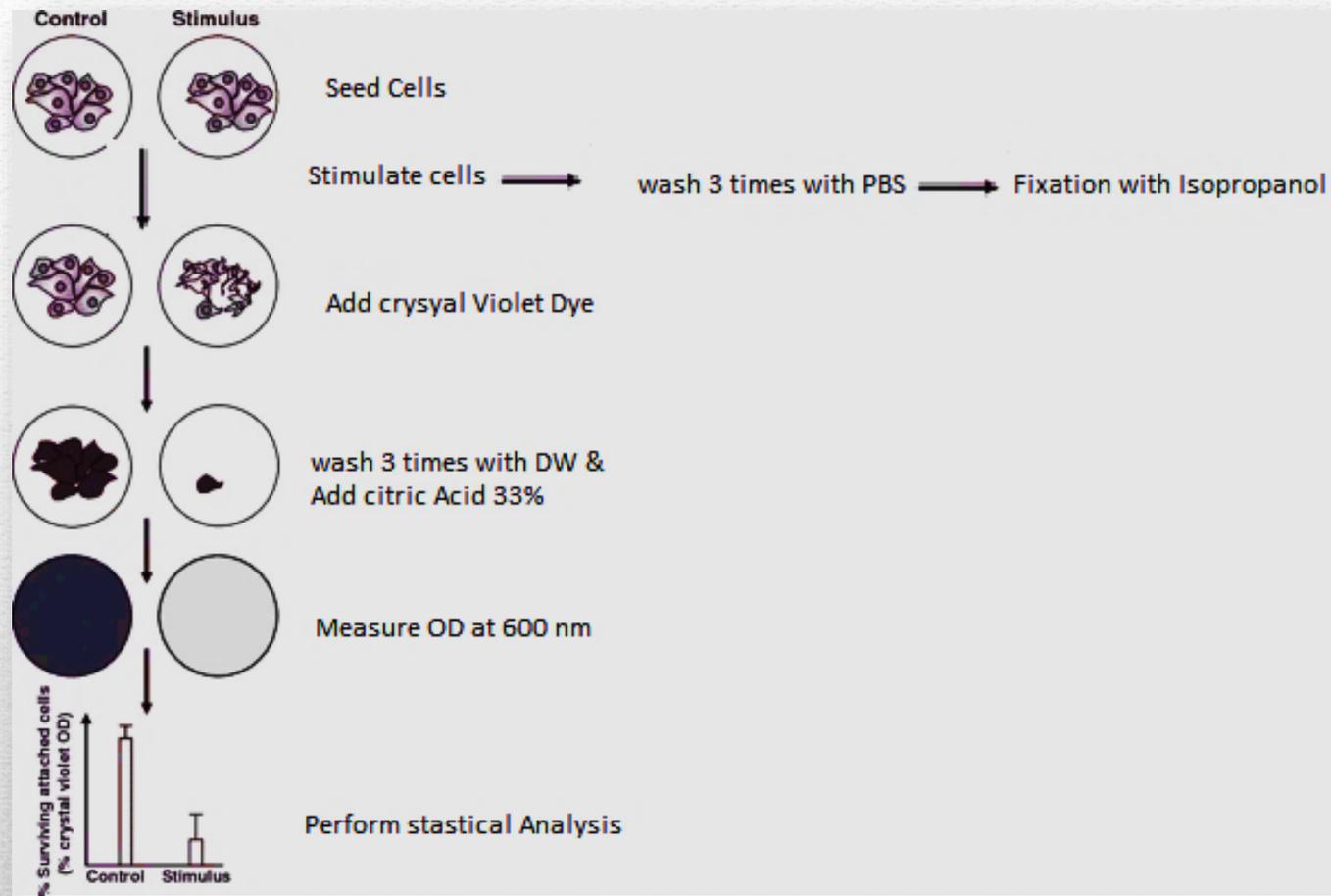


2. Isolation and Cultivation of Chondrocytes

Additive	US	+ve Co	PRP
ITS	1:100	1:100	1:100
Dexamethasone, nM	100	100	100
Ascorbic acid,mg/mL	50	50	50
TGF-b1, ng/mL		50	
IGF-1, ng/mL		50	
PRP powder, %			0.5, 1, 5

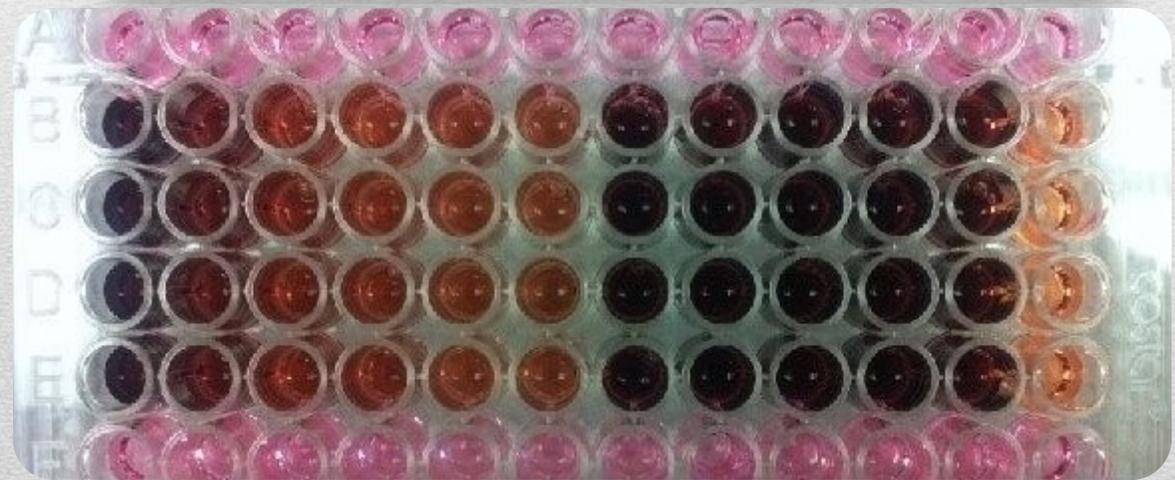
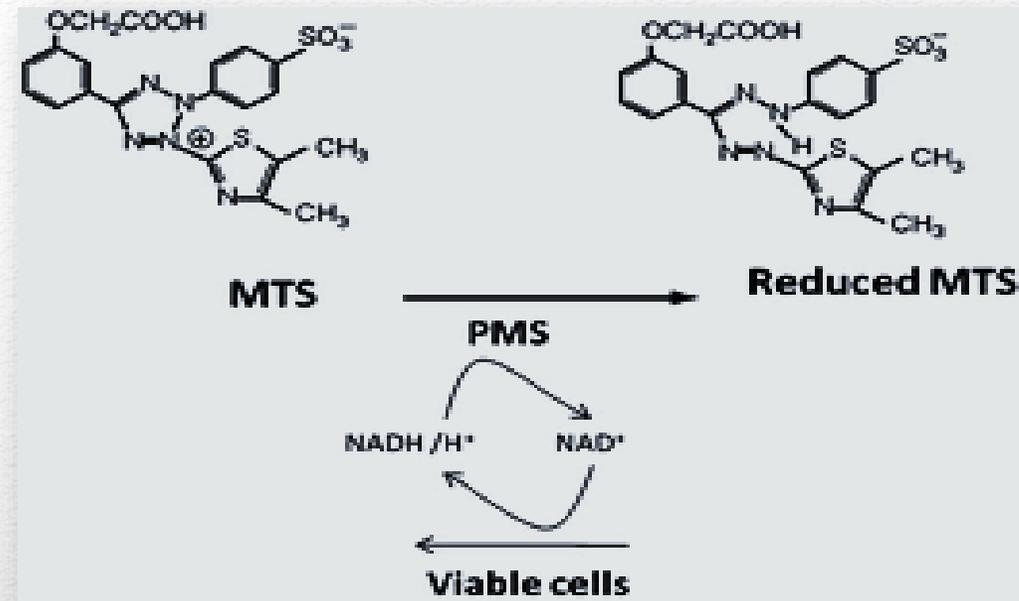
- The main component is DMEM, containing 1% penicillin/streptomycin
- The culture medium was changed on days 0, 2, 5, 7, 9, and 12

Composition of Cell Culture Media

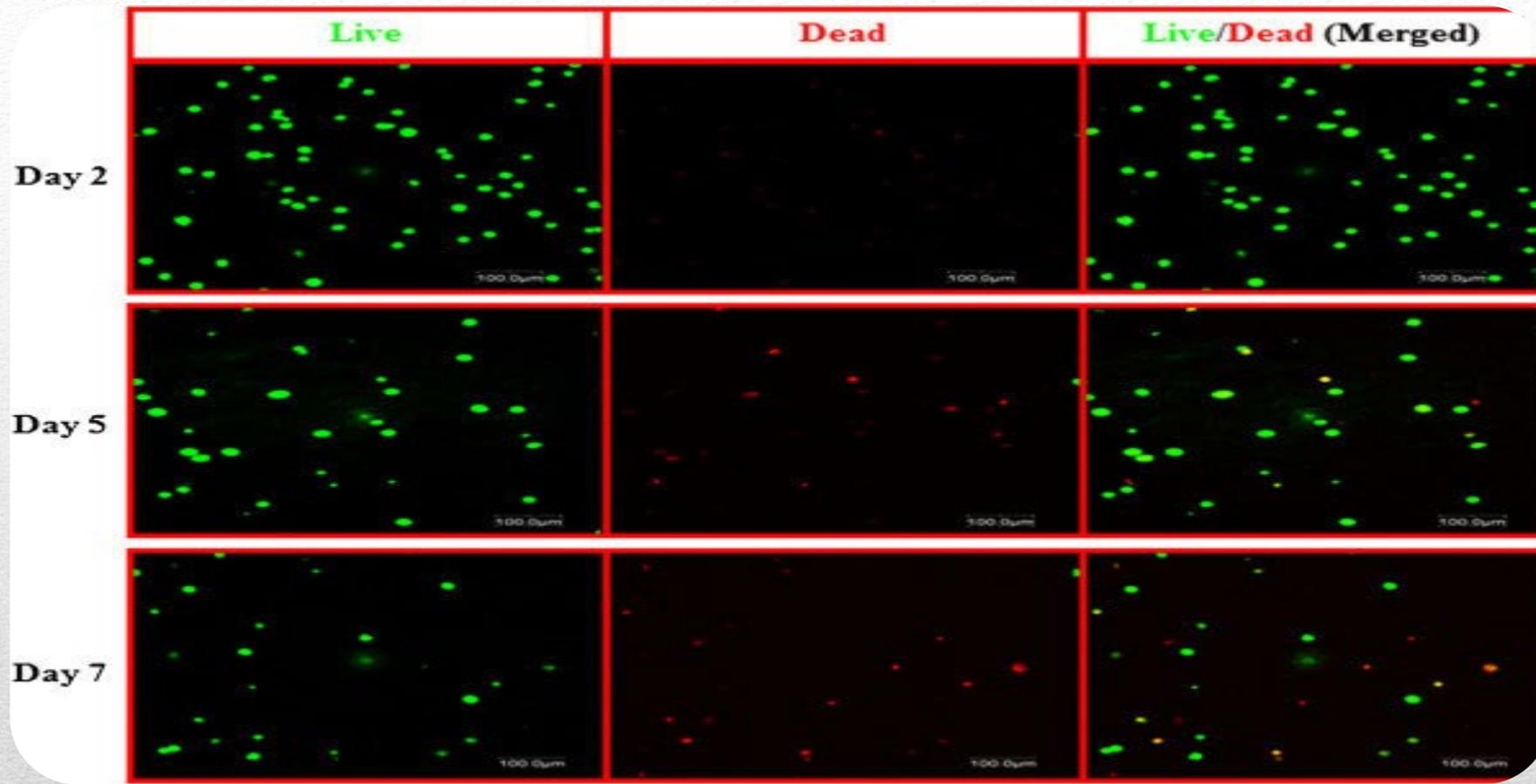


3. Crystal Violet Staining

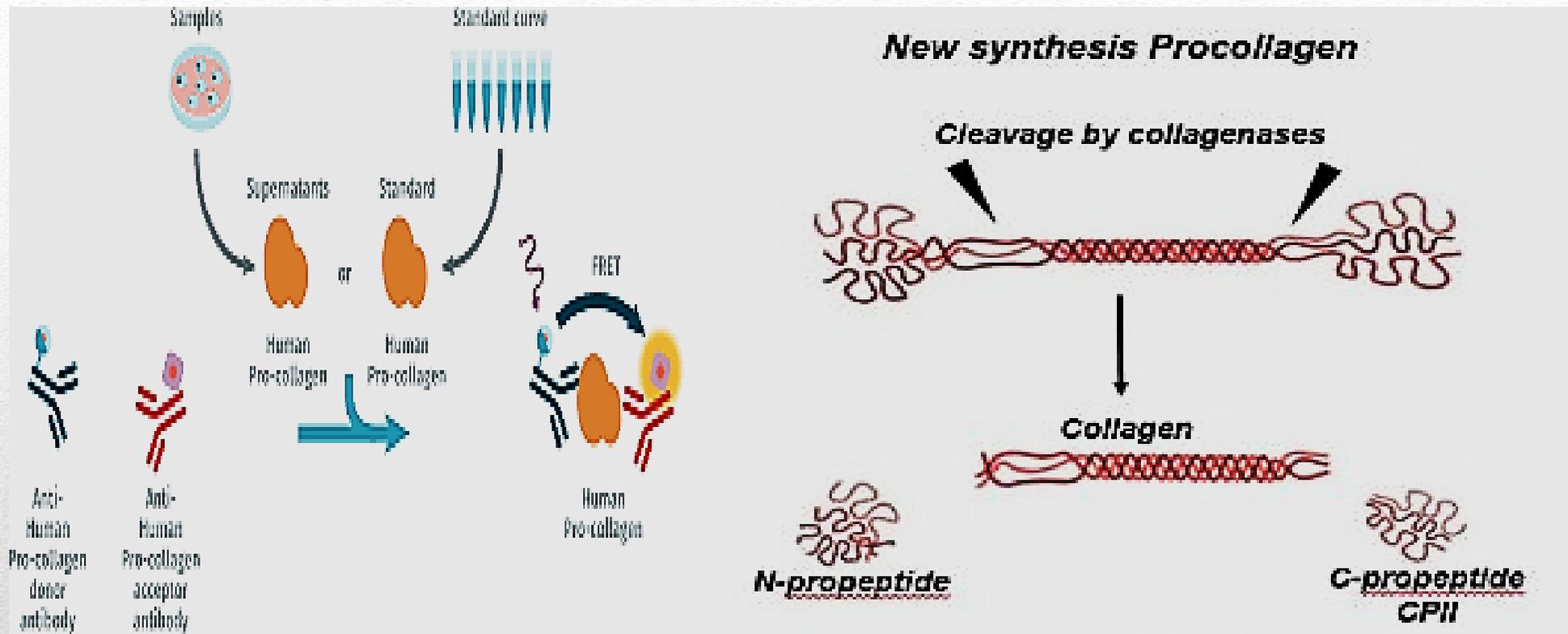
- the formazan formed from MTS is water-soluble
- less toxic
- Having a more precise sensitive absorption range
- Faster



4. MTS assay

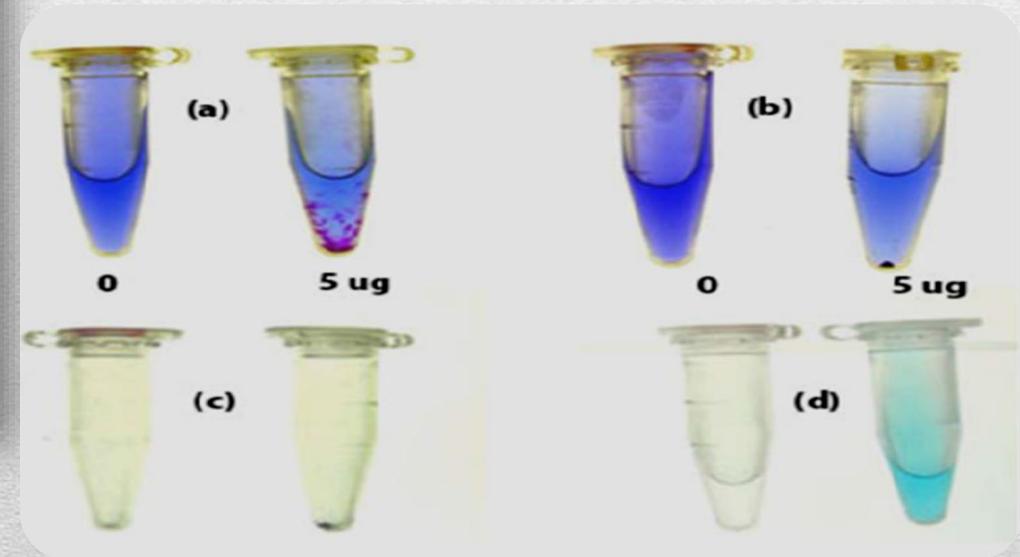
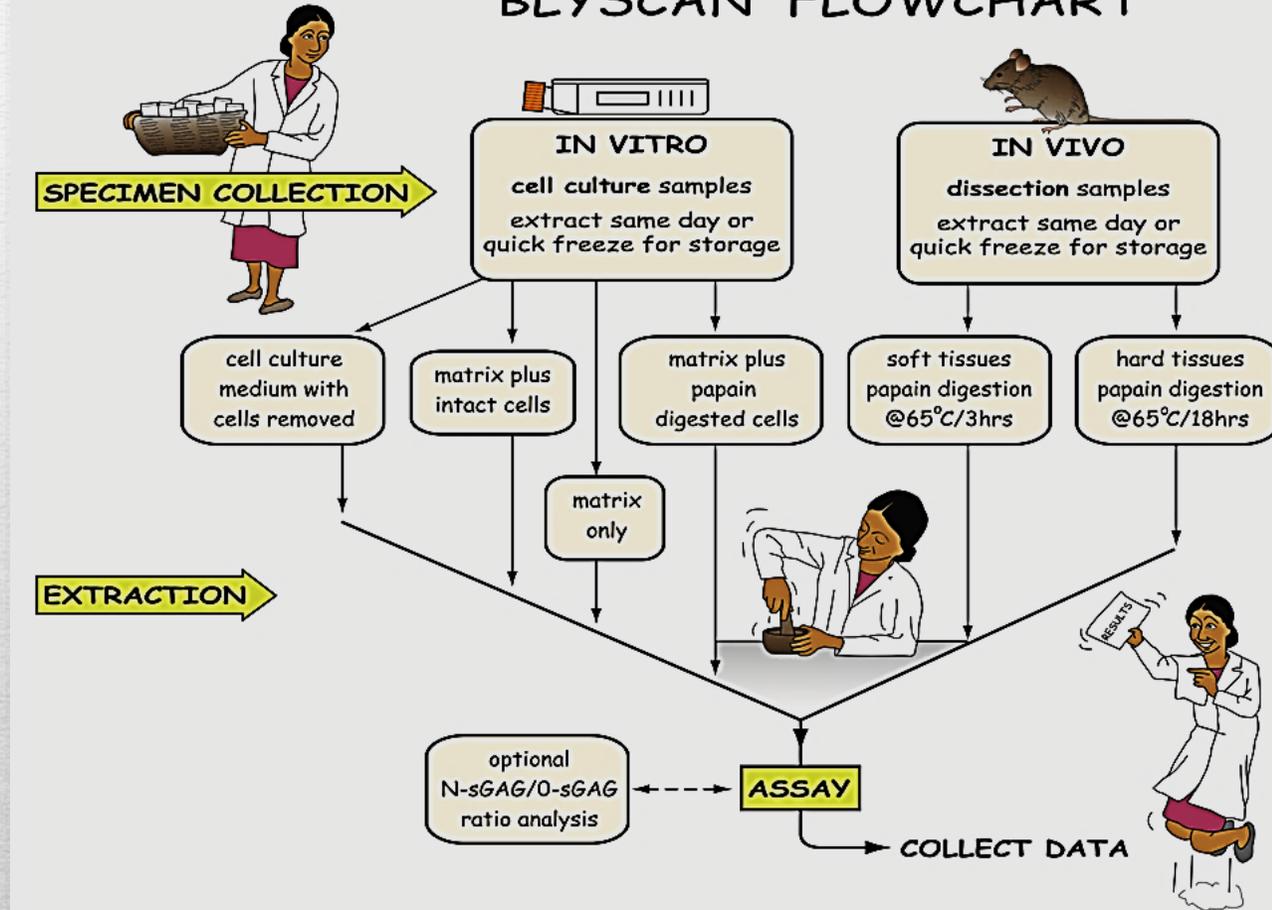


5. live-dead staining

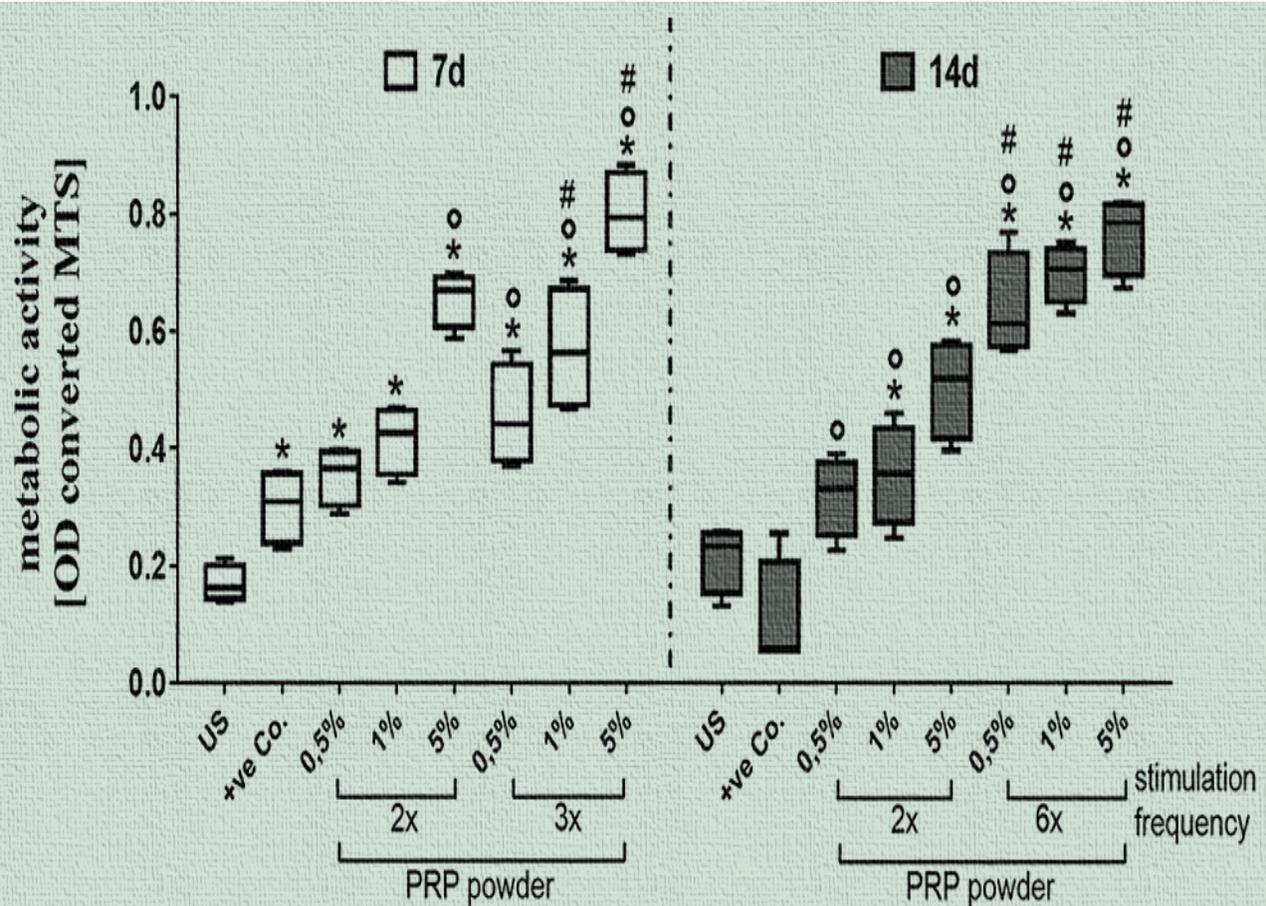
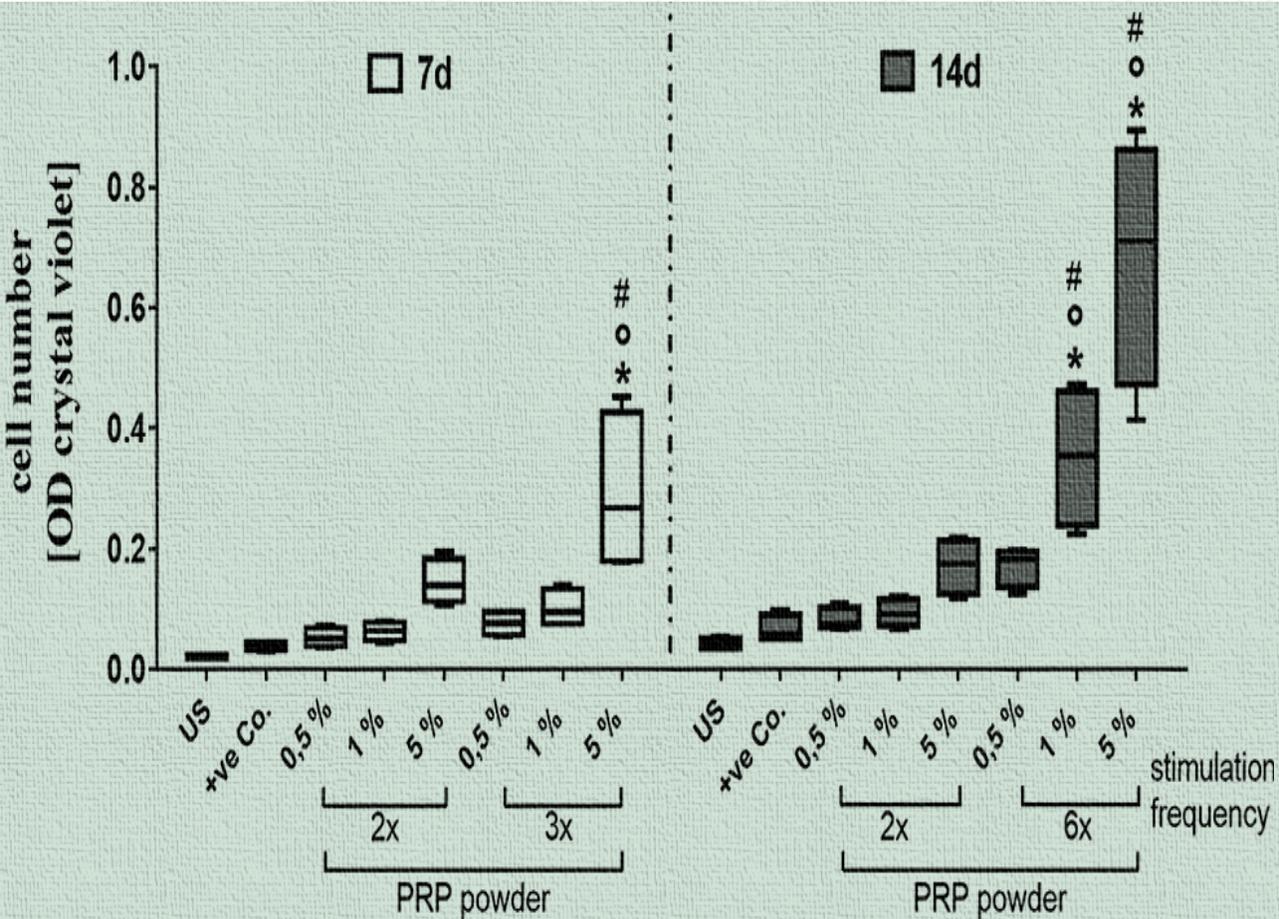


6. ELISA

BLYSCAN FLOWCHART



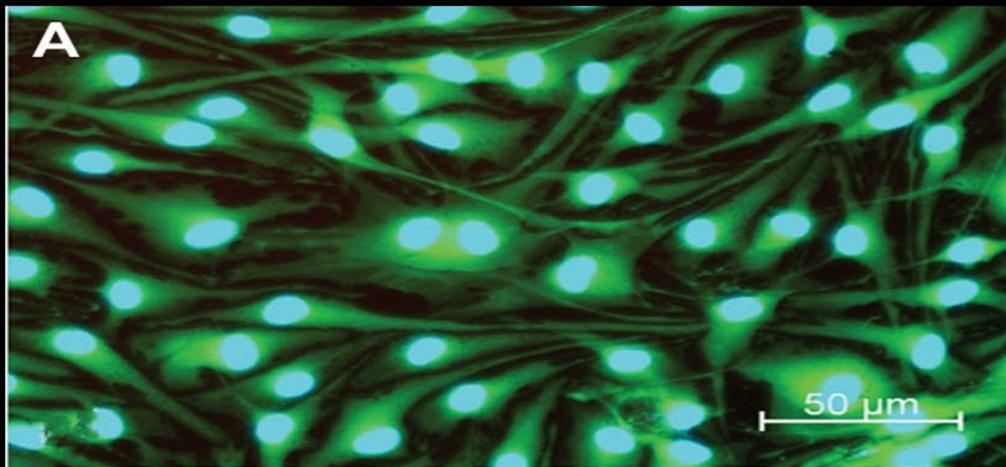
7. the Blyscan Assay



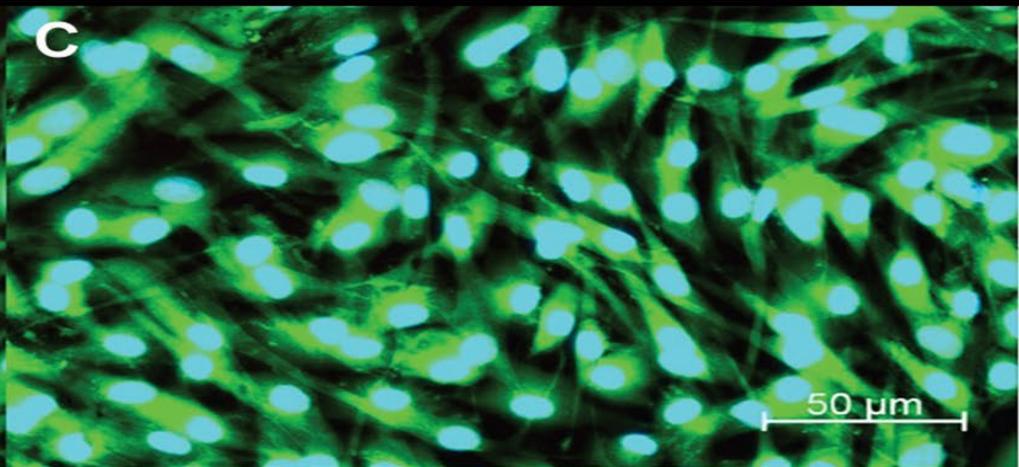
RESULTS: 1. Cell Proliferation and Metabolic Activity

US

A

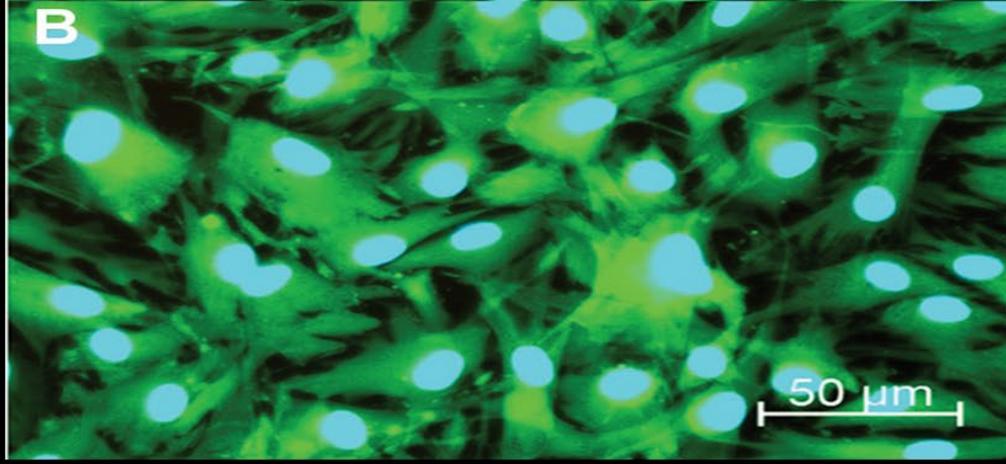


C

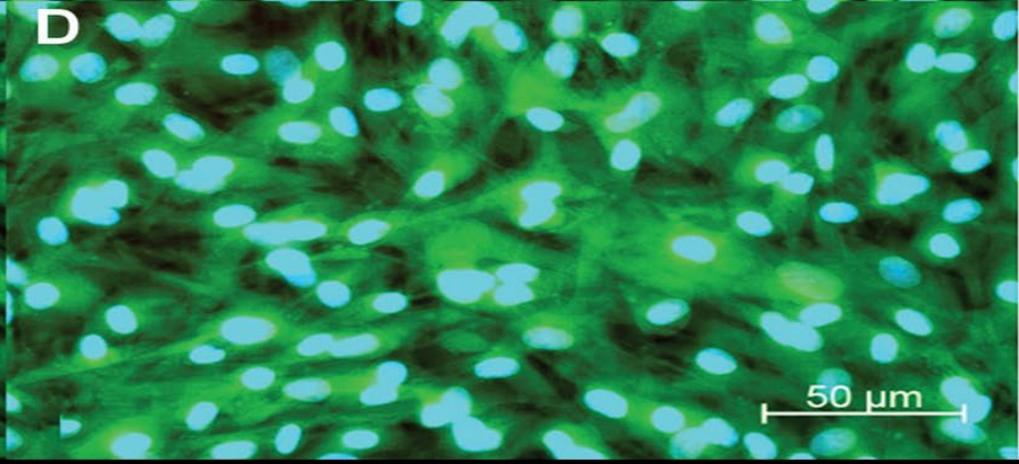


2x

B



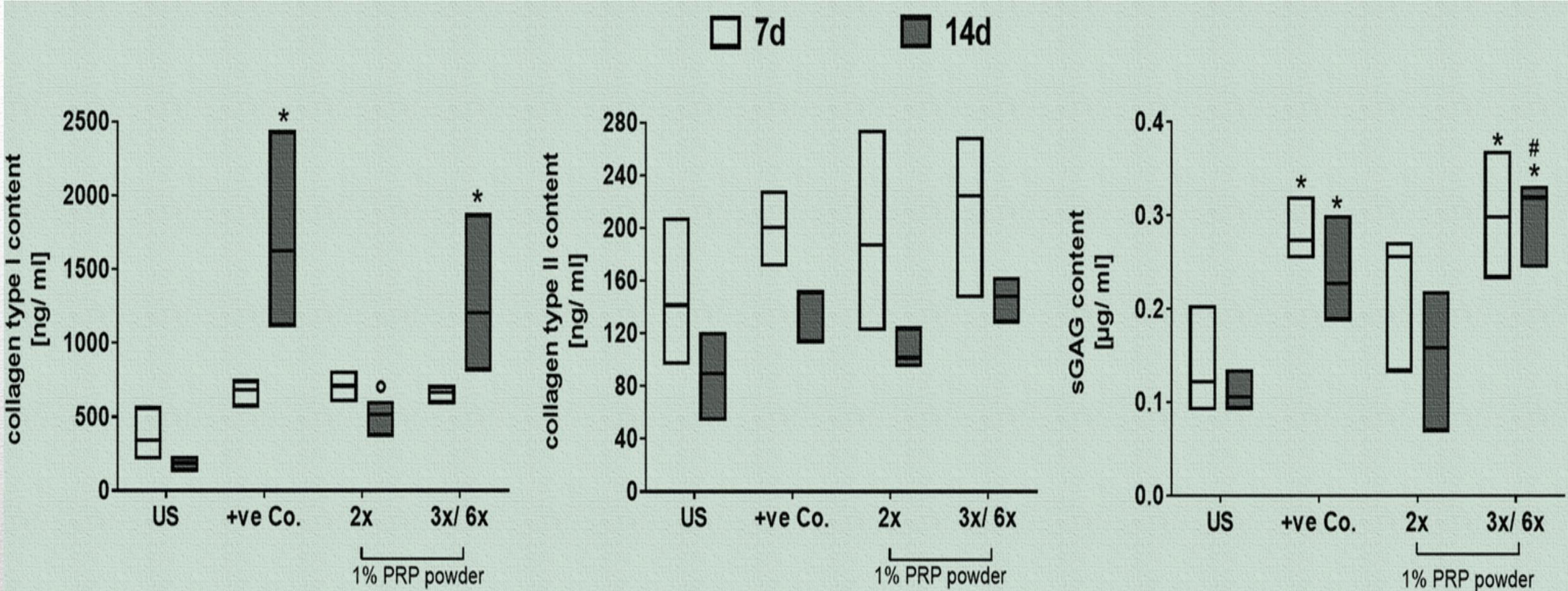
D



3x

+ve Co.

2. Cell Viability and Morphological Phenotype



3. Quantification of Chondrogenic Differentiation

- the concentration and stimulation frequency of PRP powder directly influenced the proliferation and metabolic activity of chondrocytes in a dose-dependent manner
- Isolating chondrocytes from their 3-dimensional environment results in dedifferentiation into a fibroblastic phenotype
- Optimal redifferentiation might be further achieved by approximating physiological conditions, such as an additional 3-dimensional environment or a reduction of oxygen partial pressure



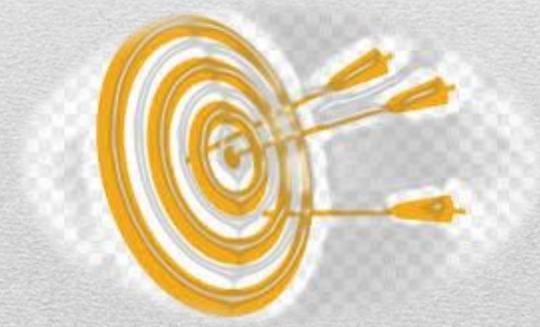
DISCUSSION

- there are differences between platelet lysates and PRP
- fewer injections with higher concentrations might be favorable
- the concentrations used in this cell culture model cannot be directly transferred to the human situation. dose-effect studies should be conducted with an animal model



Limitations of the Study

These results indicated Problems with PRP, such as the absence of standardization, the lack of consistency among studies, and the black box dosage , could be solved by using characterized PRP powder made by pooling and lyophilizing multiple platelet concentrates



CONCLUSION

