IMPACT OF ^{99m}Tc-LABELLING ON BINDING AND VIABILITY OF HUMAN ADIPOSE TISSUE-DERIVED CELLS IN VITRO AND ON THE CELL-TRACKING PROPERTIES USING SPECT IN VIVO

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Purpose

There is an emerging need for innovative approaches to augment and repair musculoskeletal tissues[1]. Stem cells derived from different sources hold a great potential as novel therapeutic approach in several diseases. Adipose tissue contains a population of mesenchymal stem cells known as human adipose tissue-derived cells (HATDCs)[2].

Fig. 1: HATDCs can be harvested readily, safely and are capable of differentiating into other mesenchymal tissue types, including not only adipocytes, myoblasts and endothelial cells but also osteoblasts and chondrocytes (BonusBio group Ltd.)

In bone tissue engineering the scaffold plays an important role as temporary support for the development of new tissue[3]. Collagen (COL) has been among the most widely used biomaterials for biomedical applications.

Here, we have set out to radiolabel COL Type I, extracted from rat tail, modified with magnetic nanoparticles or not (COL-MNPs and COL-Free, respectively) with the most widely employed SPECT radioisotope Technetium-99m (^{99m}Tc).

Method-Material

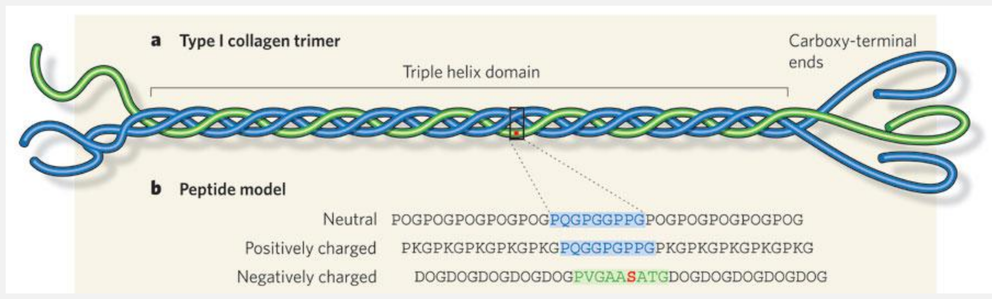


Fig. 2: Structure of type I COL, a heterodimer protein composed of two α1(I) polypeptide chains and one α2(I) polypeptide chain, which spontaneously form a triple helix scaffold at pH=7 and at 37° C, manually extracted from rat tail tendon, at a final concentration of 4.0 (mg/ml) in acetic acid, performed by Bioimag following a protocol suggested by Advanced BioMatrix, Inc..

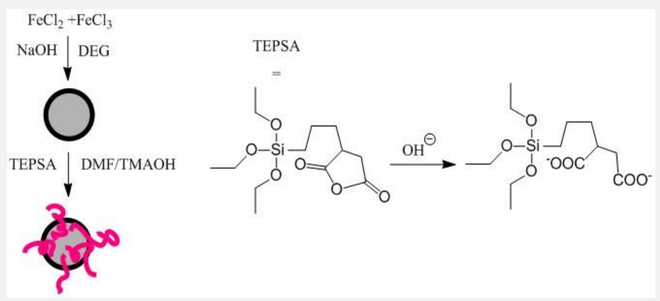


Fig. 3: Synthetic route to obtain MNPs: i) co-precipitation of iron salts in basic organic medium; ii) stabilization of the iron oxide cores by means of TEPSA treatment according to a published protocol by UMONS[4]

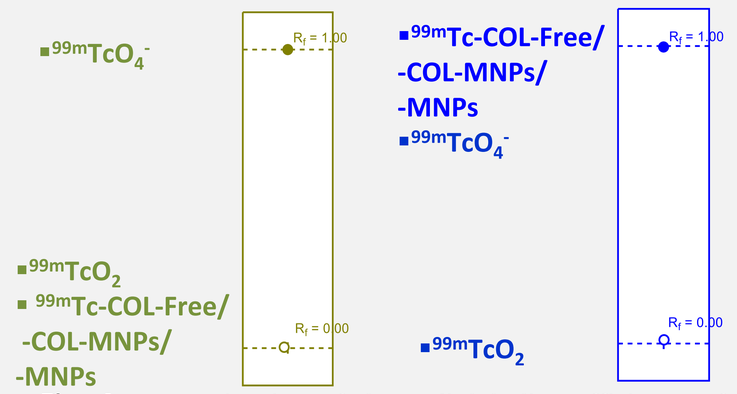


Fig. 4: Determination of the radiolabeling efficiency of both forms of COL solution and of MNPs and of the radiochemical purity performed by ITLC – SG using acetone and a mixture of pyridine: acetic acid: water (3:5:1.5) as the mobile phases[5]

Results

Table 1: COL gelation (fiblrillogenesis) was performed at two different concentrations of 1.0 (mg/ml) and 3.0 (mg/ml) in a final volume of 1.0 ml. The volume ratio of the reagents used is summarized below.

	Concentration	
Reagents	**1.0 (mg/ml)**	**3.0 (mg/ml)**
Collagen	**0.25 ml**	**0.75 ml**
(10X) PBS	**0.1 ml**	**0.1 ml**
1N, NaOH	**0.0045 ml**	**0.01275 ml**
dH_2O	**0.64 ml**	**0.13 ml**

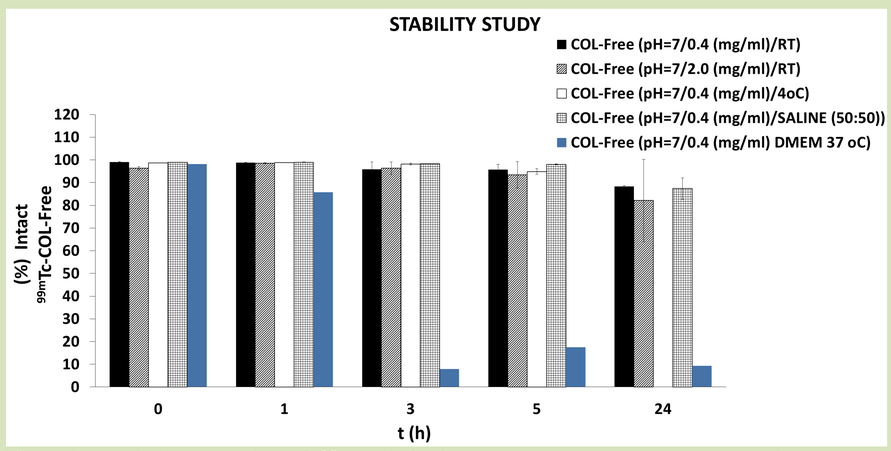


Fig. 5: Stability study of ^{99m}Tc-labeled COL-Free solution in relation to: concentration (0.4 (mg/ml) and 2.0 (mg/ml)); temperature (RT and 4 °C); in the presence of an isotonic solution (NaCl (0.9 (%)); culture media at 37 °C, performed at NCSR "Demokritos", Greece

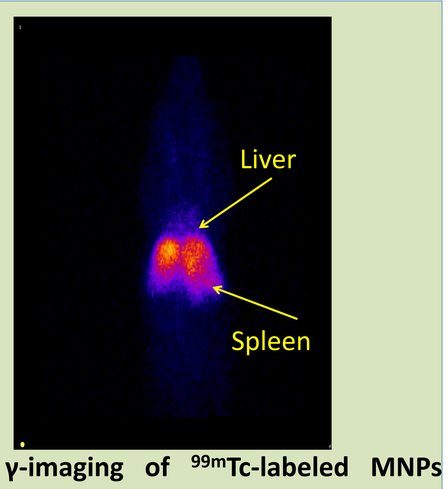
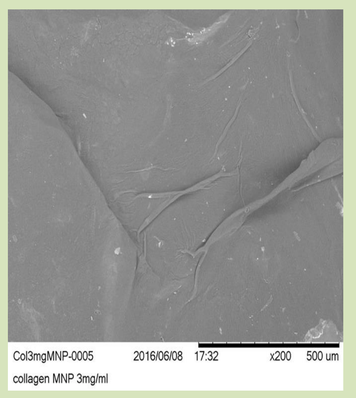


Fig 6. Planar γ-imaging of ^{99m}Tc-labeled MNPs (100ul, 100uCi or 3.7MBq) at 1h p.i. in Albino Swiss Webster mice by a NanoSPECT/CT system (Mediso) installed at the Centre for Microscopy and Molecular Imaging (CMMI), Belgium



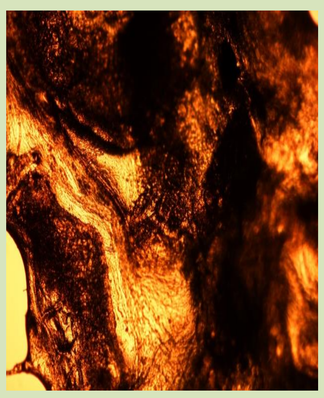


Fig. 7: SEM image, at only 200 × magnification area, of COL–MNPS 3D scaffolds Scale bar indicates 500 μm (left); Light microscope image of COL–MNPS 3D scaffolds (right) (preliminary results taken at CSIC, Spain)

Fig. 8: In vivo MRI data collected on Albino Swiss Webster mice injected with: ^{99m}Tc-labeled MNPs (three images aligned to the left side); MNPs (three images aligned to the right side) by a 9.4T Biospec (Bruker) installed at the Centre for Microscopy and Molecular Imaging (CMMI), Belgium; Circles are drawn around the liver (green circle), spleen (yellow circle) and regions selected around the bone (yellow area)

Conclusion

- ✓ **The radiolabeling yield is high in all studied samples (COL-Free, COL-MNPs and MNPs) as well as the stability of studied COL-Free in aqueous media up to 24 post-preparation independently of the temperature kept and the concentration tested**
- ✓ **3D gel preparation of COL (COL-Free and COL-MNPs) was successfully performed at two different concentrations under aseptic conditions**
- ✓ **In SEM image some big bundles of COL-MNPs fibrils lying around can be observed although the magnification isn't so high to see single COL fibrils which look better by light microscope**
- ✓ **The in vivo uptake of labeled or non-labeled MNPs (via γ- and/or MRI imaging) shows significant uptake in the mononuclear phagocytic system (MPS) such as liver, spleen, bone marrow**
- ✓ **HATDCs successful labelling, uptake kinetics and cell viability (affected by radiolabeling) studies are still ongoing**

References

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Acknowledgment

This research is carried out in terms of the H2020-MSC-RISE project VIVOIMAG (645757)

5th Balkan Congress & 13th National Congress of Nuclear Medicine, 17-20 June 2016 Makedonia Palace Hotel, Thessaloniki, Greece