# Collagen scaffolds labelled with MRI and SPECT contrast agents for non-invasive imaging in bone tissue engineering


*Fragogeorgi Eirini[1], Daich Julian[2], Fysikopoulos Eleftherios[3], Lacroix Simon[4], Doumont Gilles[4], Georgiou Maria[3], Stanicki Dimitri[5], Pita Marcos[6], Laurent Sophie[4,5], Bouziotis Penelope[1], Velez Marisela[6], Loudos George[1,7]*

*[1]National Center for Scientific Research (NCSR) "Demokritos", Aghia Paraskevi - Athens, Greece*
*[2]Bioimag Soluciones de Contraste, S.L., Caceres, Spain*
*[3]BioEmission Technology Solutions, Athens, Greece*
*[4]Center for Microscopy and Molecular Imaging (CMMI), Belgium*
*[5]University of Mons, Mons, Belgium*
*[6]Spanish National Research Council (CSIC), Madrid, Spain*
*[7]Technological Educational Institute of Athens (TEIA), Greece*


## Introduction

**Non-invasive imaging gains interest in the evaluation of novel synthetic scaffolds in bone tissue engineering as an alternative approach to the clinical gold standard treatment (autografting)[1]. Herein, we employ collagen-based scaffold materials as bone implants labelled with magnetic nanoparticles (MNPs)[2,3] (MCF) and with $^{99m}$Tc[4] (CF) for *in vitro* detection of enzymatic activity of metalloproteases[5]. This labelling will further permit to *in vivo* follow the fate of these new bone grafts supplemented with cells, using MRI, nuclear imaging and CT[6].**

## Methods

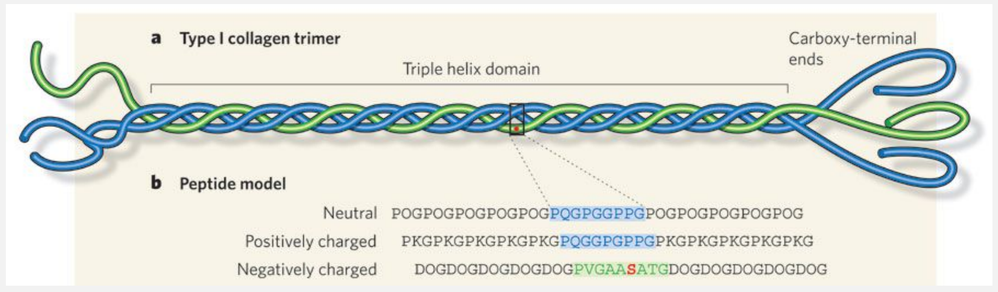



**Fig. 1: Structure of type I collagen, a heterodimer protein composed of two α1(I) polypeptide chains and one α2(I) polypeptide chain, which spontaneously form a triple helix scaffold at pH=7 and at 37º C, manually extracted from rat tail tendon, at a final concentration of 4.0 (mg/ml) in acetic acid.**

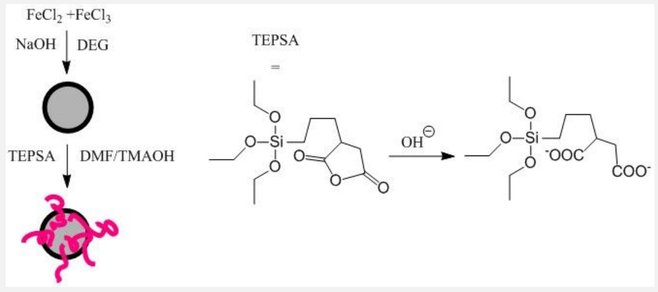



**Fig. 2: Synthetic route to obtain MNPs: i) co-precipitation of iron salts in basic organic medium; ii) stabilization of the iron oxide cores by means of TEPSA treatment [2]**

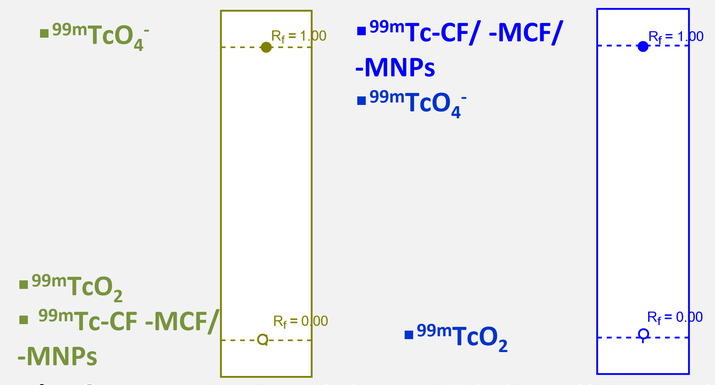



**Fig. 3: Determination of the radiolabeling efficiency of both forms of collagen (CF, MCF) and of MNPs and of the radiochemical purity performed by ITLC – SG using acetone and a mixture of pyridine: acetic acid: water (3:5:1.5) as the mobile phases[4]**

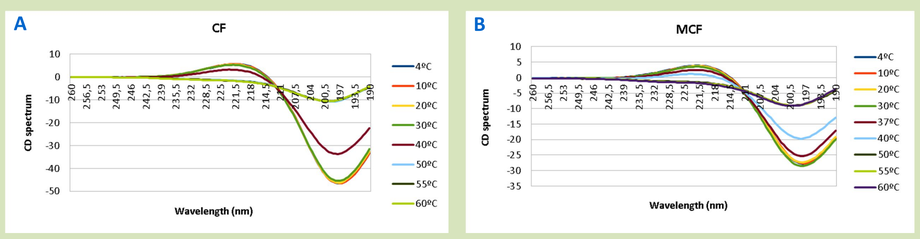



**Fig. 4: Variation of CD spectrum of unmodified collagen (CF) (A) and of MNP-labelled collagen (MCF) (B) as a function of the temperature. The min and max values for CF were observed at 198.06 nm ±1.14 and at 223.25 nm ±2.25; The min and max values for MCF were observed at 198.5 ±1.14 and at 222.77 ±1.73 respectively. The collagen samples concentration was 0.1 mg/mL in acetic acid.**

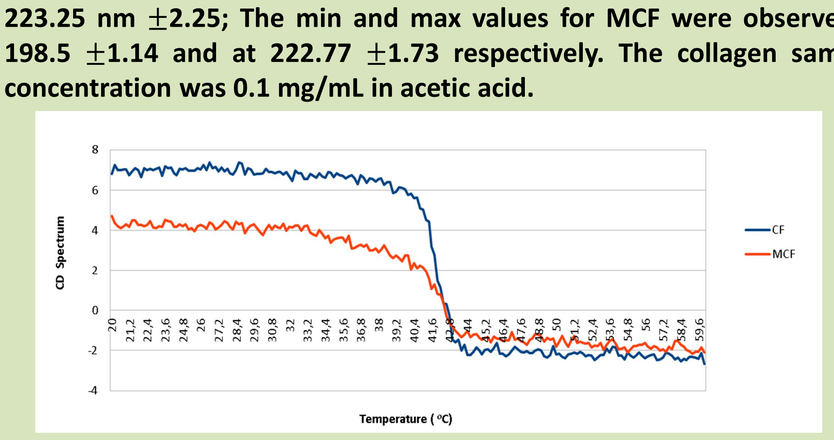
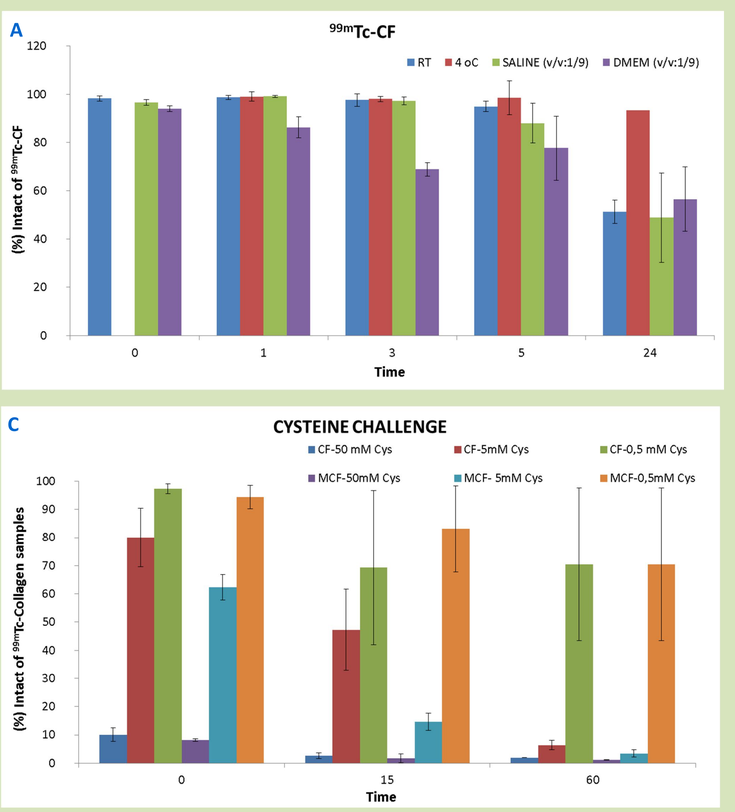



**Fig. 5: Comparative CD spectrum for CF and MCF at 221 nm. Melting effects were observed at temperatures higher than 40º C for both samples. Td values were observed at 42,7º C and 42º C for CF and MFC respectively. The collagen samples concentration was 0.1 mg/mL in acetic acid.**



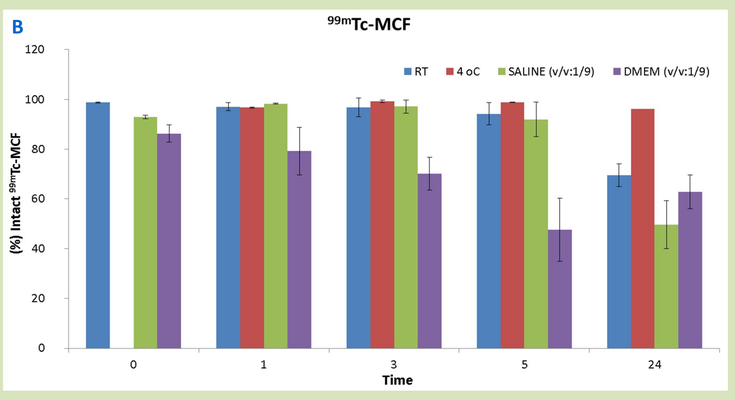




**Fig. 6: Stability study of: (i) $^{99m}$Tc-labeled CF (A) MCF (B) in relation to temperature (RT and 4 ºC); in the presence of an isotonic solution (NaCl (0.9 (%)); culture media at 37 ºC; (ii) both labelled collagen samples (C) in the presence of excess amount of cysteine.**

| | (%) Collagenolytic radioactivity (CF) | (%) Collagenolytic radioactivity (MCF) |
|---|---|---|
| t=0 | 21.9±7.8 | 11.6±3.8 |
| t=2h | 61.2±26.1 | 44.0±1.1 |
| t=4h | 87.2±9.9 | 87.4±5.5 |

**Table 1: Collagenase assays following incubation of collagenase (1.0 mg/mL) with the radiolabeled collagen preparations used as substrate at 37ºC.**

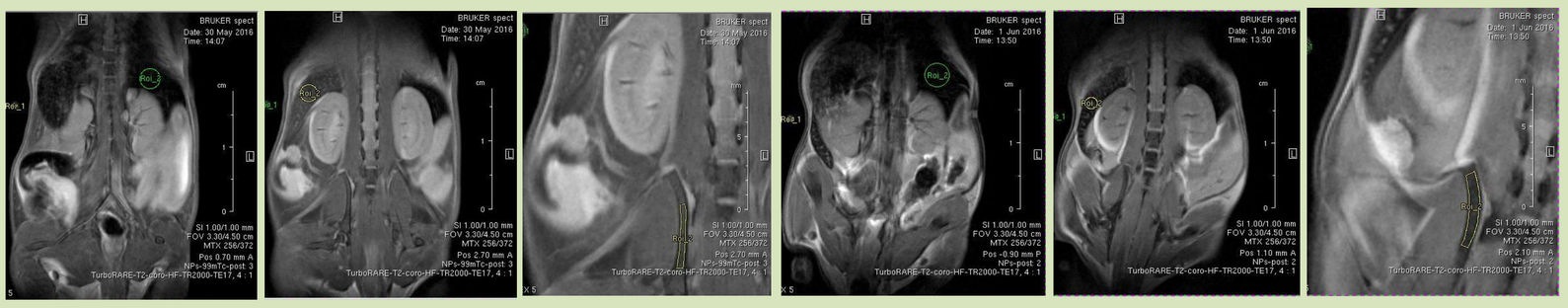



**Fig. 7: *In vivo* MRI data collected on Albino Swiss Webster mice injected with: $^{99m}$Tc-labeled MNPs (three images aligned to the left side); MNPs (three images aligned to the right side) by a 9.4T Biospec (Bruker) ; Circles are drawn around the liver (green circle), spleen (yellow circle) and regions selected around the bone (yellow area)**

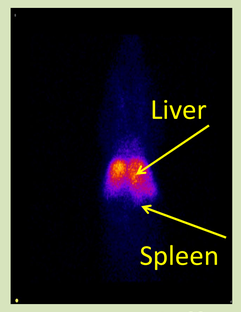



**Fig 8. Planar γ-imaging of $^{99m}$Tc-labeled MNPs (100ul, 100uCi or 3.7MBq) at 1h p.i. in Albino Swiss Webster mice by a NanoSPECT/CT system (Mediso)**

## Conclusions

- ✓ **CD curves were similar for collagen free (CF) and magnetically modified collagen (MCF). Denaturalization was clearly when <40 ºC.**
- ✓ **No significant difference was observed between CF and MCF monitoring the radiolabeling stability tests.**
- ✓ **A linear increase in collagenolytic activity occurred over time for both radiolabelled collagen samples.**
- ✓ ***In vivo* uptake of labeled or non-labeled MNPs was significant in the mononuclear phagocytic system.**
- ✓ **Colorimetric experiments of collagen degradation after being exposed to collagenase over time are ongoing.**
- ✓ **Animal models are now established and imaging results encourage in vivo monitoring of MCF or cell/MCF constructs-induced inflammation, enzyme activity and bone formation in mouse models with calvarium bone defects using MRI and SPECT/CT[6].**

## References


1. M. Ventura, O. C. Boerman, C. de Korte, M. Rijpkema, A. Heerschap, E. Oosterwijk, J. A. Jansen, X. F. Walboomers, Tissue Eng. Part B, Rev., 1-18, (2014)
2. D. Stanicki, S. Boutry, S. Laurent, L. Wacheul, E. Nicolas , D. Crombez, L. V. Elst, D. L.J. Lafontaine, R. Muller, J. Mater. Chem. B, 2, 387-397, (2014)
3. M. Pita, J. M. Abad, C. Vaz-Dominguez, C. Briones, E. Mateo-Martí, J. A. Martín-Gago, M. del Puerto Morales, V. M. Fernández, J.Colloid Interface Sci., 321, 484-492 (2008)
4. E. A. Fragogeorgi, I. N. Savina, T. Tsotakos, E. Efthimiadou, S. Xanthopoulos, L. Palamaris, D. Psimadas, P. Bouziotis, G. Kordas, S. Mikhalovsky, M. Alavijeh, G. Loudos, Int. J. Pharm. 465, 333-346 (2014)
5. T. E. Cawston, Nat. Protoc. 4(3), 286-290 (2009)
6. P. S. Lienemann, S. Metzger, A-S Kiveliö, A. Blan, P. Papageorgiou, A. Astolfo, B. R. Pinzer, P. Cinelli, F. E. Weber, R. Schibli, M. Béhé, M. Ehrbar. Sci. Rep. 5, 10238; doi: 10.1038/srep10238 (2015)


## Acknowledgment


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


**12th European Molecular Imaging Meeting**
**5-7 April 2017**

European Molecular Imaging Meeting – EMIM 2017