“INTERACTION and CELL PROLIFERATION in BIOACTIVE COLLAGEN MATRICES”

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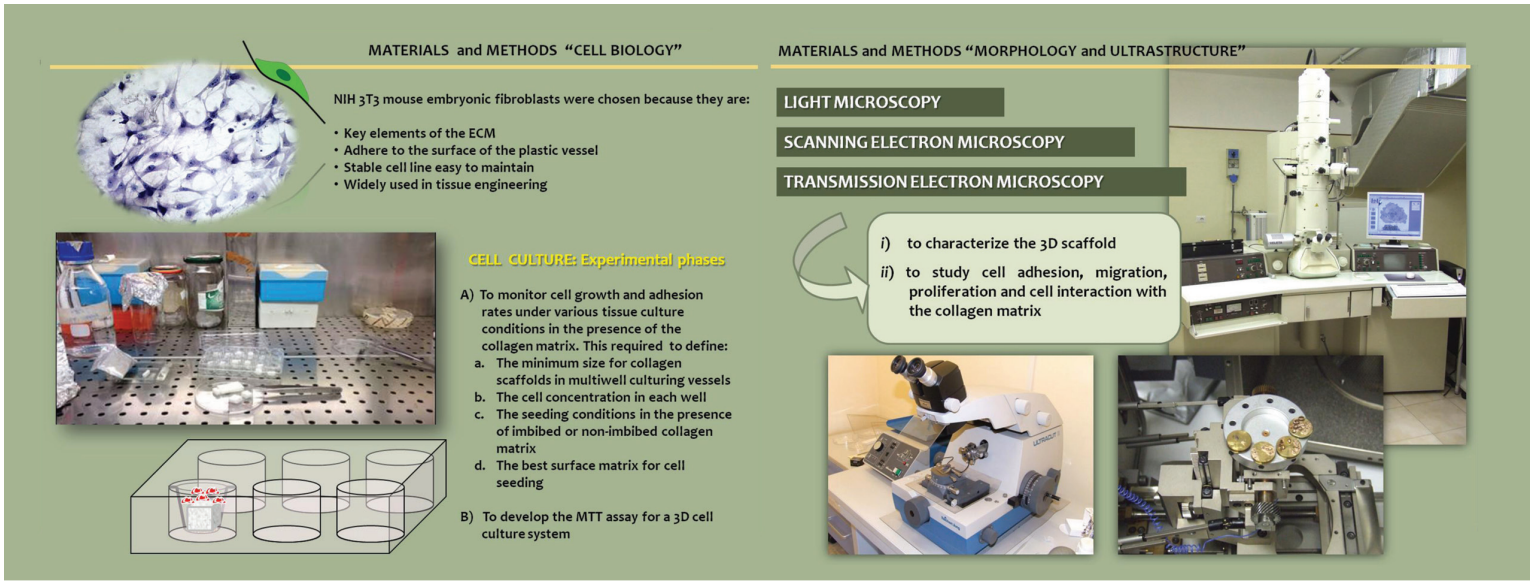
INTRODUCTION

A variety of collagen scaffolds are available today to help wound healing and skin regeneration. To accomplish these goals, scaffolds ought to satisfy several requirements.First, the pore size has to be maintained within a restricted size range for cells to adhere to the collagen matrix and migrate inside the scaffold. Second the scaffold itself should be sufficiently stable to allow new granulation tissue to mature while the inujured tissue is replaced by new collagen deposition. Third, the collagen matrix should not persist indefinitely, but be degradable within the time periods compatible with the healing process. All of these requirements were actually monitored in this study by simulating wound healing in the in vitro co-culturing system of the Biopad collagen scaffold with 3t3 fibroblasts and a comparison with promogran was made.

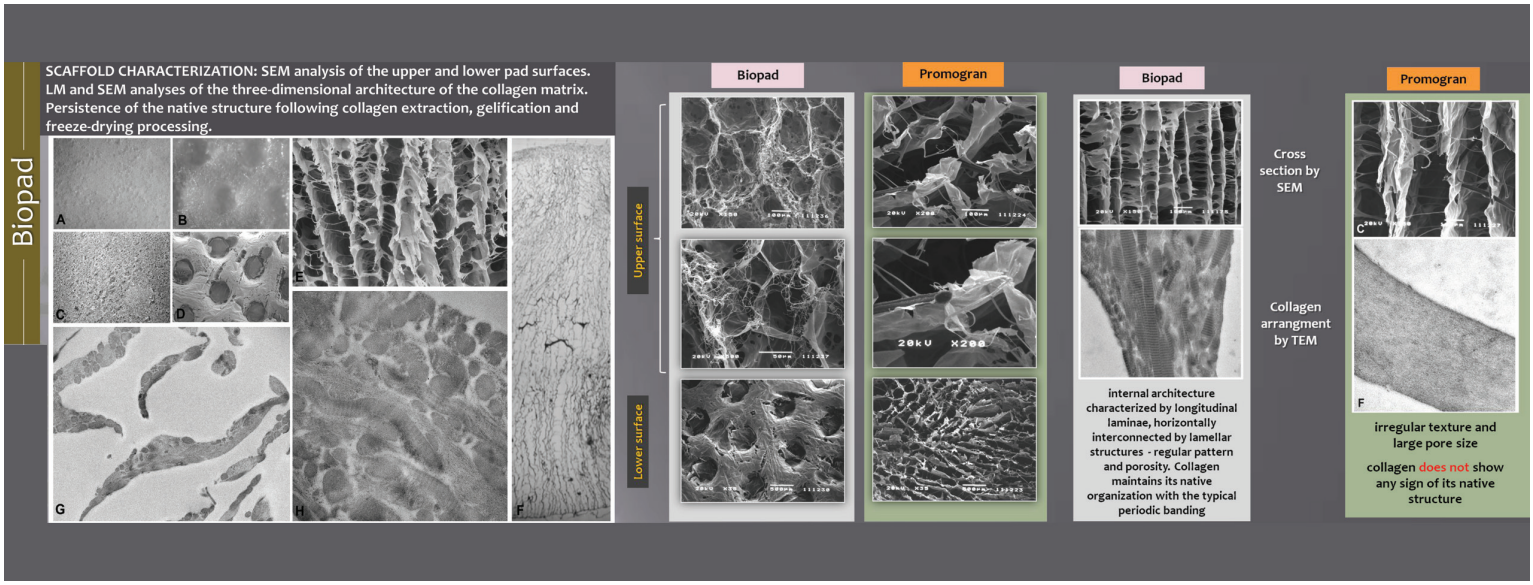


THE AIM

Analysis of the MORPHOLOGY and ULTRASTRUCTURE of EQUINE COLLAGEN TYPE 1 (Biopad) 3D scaffold

STUDIES on in vitro INTERACTION of NIH 3T3 fibroblasts with the Biopad 3D scaffold

Pure Equine collagen (Biopad) vs Collagen & Cellulose (Promogram)



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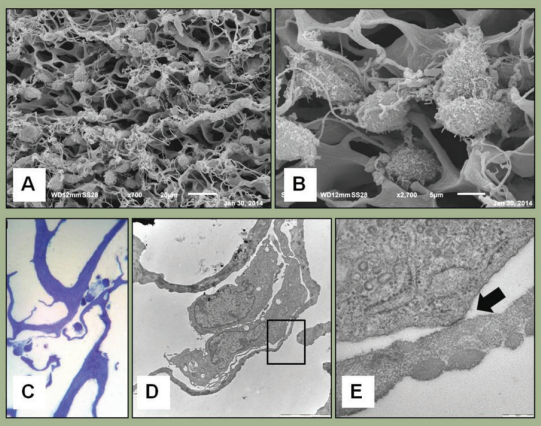
CELL/COLLAGEN MATRIX INTERACTION: adhesion and migration in Biopad 3D scaffold

4h

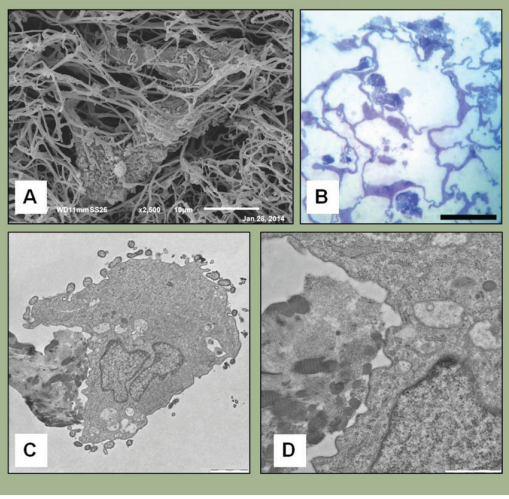


At this time point, fibroblasts have gained access to the collagen matrix. While most cells still have a roundish appearance, others have already acquired the flattened morphology, typical of migratory cells

A clear indication that cells have actually interacted with the collagen matrix is evidenced by the increased electron density of the contact points (*see arrow*)

CELL/COLLAGEN MATRIX INTERACTION: adhesion and migration in Biopad 3D scaffold

1-3 days

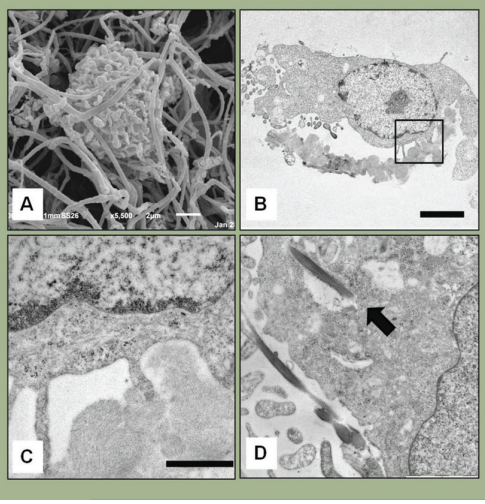


At this time point, fibroblasts have fully penetrated the collagen matrix by migrating amongst the highly intertwined fibers

The presence of vacuoles with different electron density in the fibroblast cytoplasm is taken as an indication that some collagen fragments are being digested intracellularly

CELL/COLLAGEN MATRIX INTERACTION: adhesion and migration in Biopad 3D scaffold

1-3 days

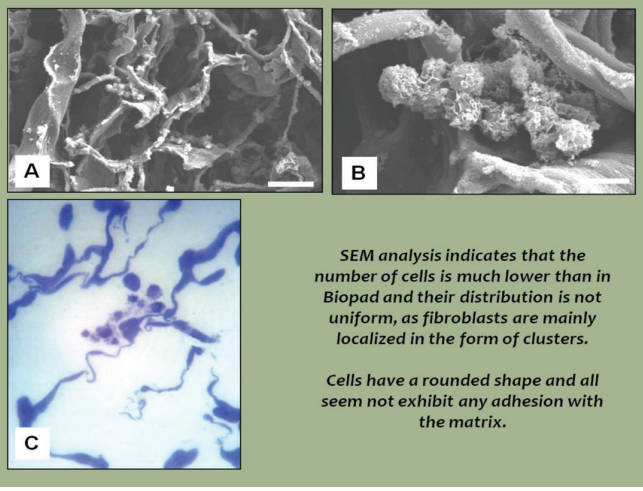


By interacting with the collagen matrix, fibroblasts protrude with their cell extensions onto the lamellae and help to disassemble the collagen fibers

The presence of collagen fibers in the fibroblast cytoplasm indicates that the extracellular matrix has been incorporated intracellularly

CELL/COLLAGEN MATRIX INTERACTION: adhesion and migration in Promogran 3D scaffold

4h

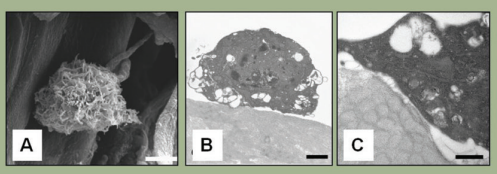


SEM analysis indicates that the number of cells is much lower than in Biopad and their distribution is not uniform, as fibroblasts are mainly localized in the form of clusters.

Cells have a rounded shape and all seem not exhibit any adhesion with the matrix.

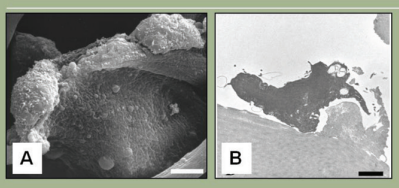
CELL/COLLAGEN MATRIX INTERACTION: adhesion and migration in Promogran 3D scaffold

1 day



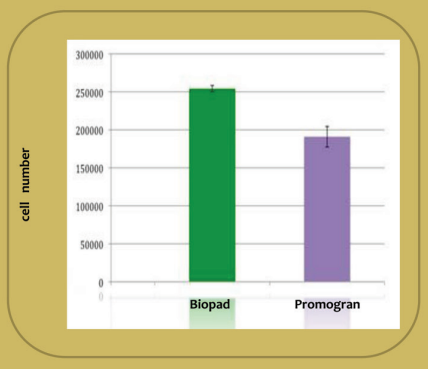
At this time point, cells are not polarized and still in contact with the collagen matrix to indicate that they have not yet started to migrate through the matrix

3 days



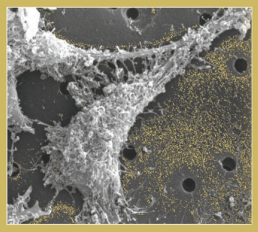
Cell activity – as expressed by such parameters as adhesion and phagocytosis – is less pronounced in this matrix than in other types of collagen scaffold

CELL ADHESION on 3D SCAFFOLDS



Cells were seeded on the Biopad upper surface (fibrillar) and on each of the two Promogran surfaces

Direct cell counting in each collagen matrix, indicates that the number of cells adhering to the Biopad scaffold is greater than that of the Promogran scaffold



Control cells: 3T3 fibroblasts cultured on 2D

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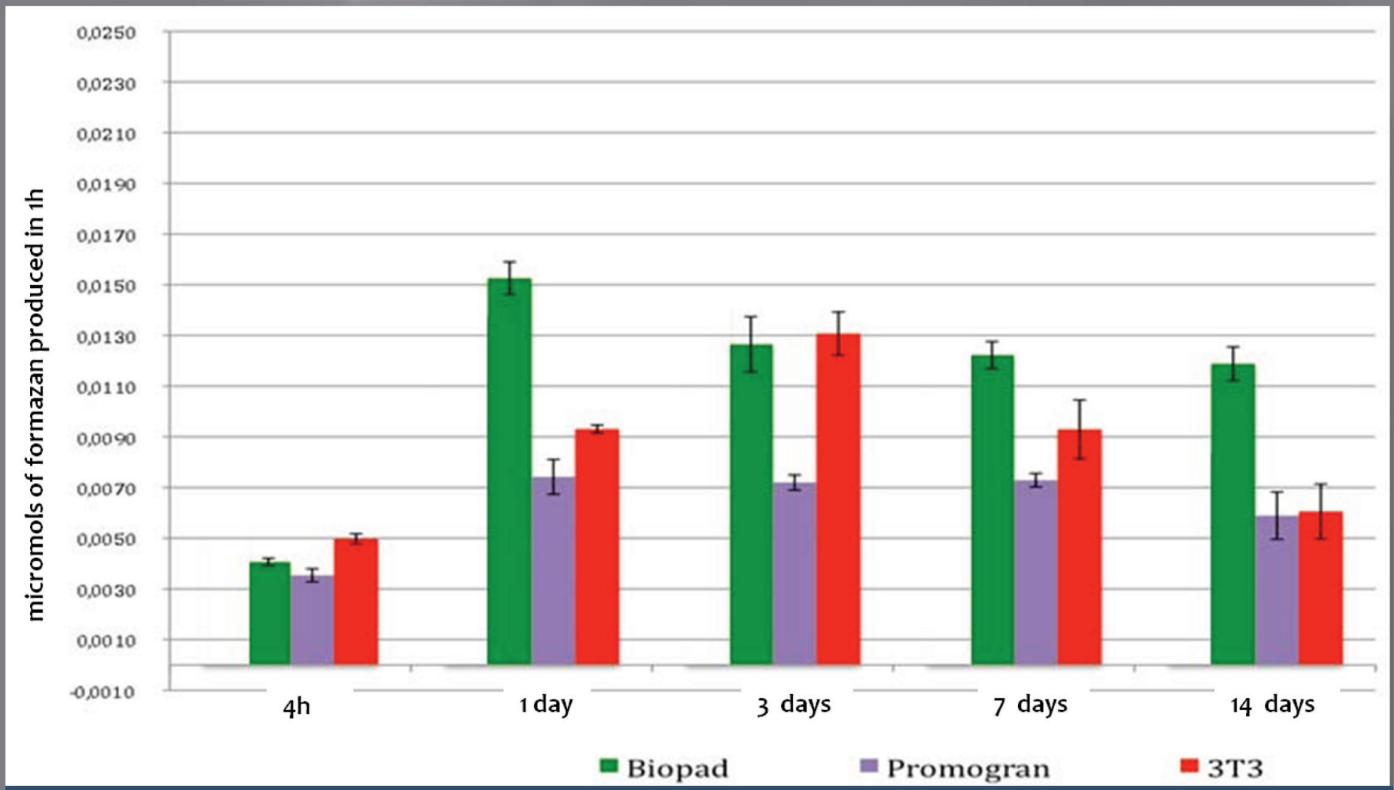
VITALITY: MTT assay



Cellular activity increases up to reach the maximum of expression at 1 day of culture. The extent of the increase differs from sample to sample. At 1 day, it is significantly greater in Biopad respect to Promogran and control (3T3 cells in 2D). In later times, Biopad shows a quite high cell activity, while in Promogran is lower and maintains the same level of the control up to 14 days.

CONCLUSIONS

Structural characteristics of the scaffold

The morpho-functional analysis has clearly demonstrated that the equine collagen Type I scaffold (Biopad) is characterized by a regularity to the size of the pores of the upper surfaces, as well as a sufficient integrity and laminar organization of internal texture. Both of these structural characteristics should be such as to sustain cell migration and to favor the conditions of interaction between the collagen matrix and the host tissue as they are close to those that are carried out *in vivo* when the support is applied on the wound.

That Biopad provides optimal conditions for cell interaction is clearly demonstrated by the fact that the 3T3 cells assume forms and behaviors of adhesion on the collagen matrix from the first time of incubation. The ultrastructural analysis has in fact allowed to document numerous instances in which cells in migration are elongated and capable of forming numerous extensions in the vicinity of the collagen matrix. When these observations were expressed in quantitative terms, it became clear that the number of adherent cells is much higher in Biopad than in Promogran. By contrast, for the same incubation time, the cells co-incubated with the Promogran still appear rounded and not able to migrate significantly.

Among the factors that favor, more than others, wound healing include in fact the proper fluids absorption and cell migration from the surrounding areas. It is obvious that a good surface porosity of collagen is a necessary, though not sufficient , because the blood fluid can be absorbed and removed with a certain efficiency , thus contributing to increase the chances of recovery , especially in the case of chronic wounds

CONCLUSIONS

Cell interactions with the scaffold

Moreover, the presence of regular pores of a size between 100 and 150 uM is such as to facilitate the efficient cell adhesion on the upper surfaces (thus avoiding the dispersion) followed by an equally effective migration in the inner zones of the support (Doillon *et al.* , 1984; Doillon and Silver, 1986; Park *et al.*, 2003; Lin and Liu, 2007; Rhee, 2009; Davydenko *et al.*, 2010). Already starting from 4h and for progressively longer times, the interaction between the matrix and the 3T3 cells becomes more pronounced and extended. In these culture conditions, at 1-3 days the cells have numerous prolongations closely interconnected with the collagen fibers, as well as the phenomena of exo-endocytosis along the plasma membrane.

Of the two collagen scaffolds compared in this study, Biopad simulates better than Promogran such cell processes as adhesion to the substrate, interstitial migration and uptake from the extracellular milieu, features that are well known to characterize wound healing *in vivo*.

Amongst the activities examined in this study, cell proliferation is perhaps most important. To this regards, it is interesting to note that the MTT assays has shown that Biopad proved better than Promogran to maintain cell viability throughout the time period tested in this study.