IJAE
Vol. 118, n. 2: 204-210, 2013
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Review in Histology and Cell Biology

New perspectives in the treatment of cartilage damage. Poly(ethylene glycol) diacrylate (PEGDA) scaffold. A review

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Submitted November 28, 2012; accepted revised April 3, 2013

Summary

This review was conducted as a complementary study to our review "Current concepts in the treatment of cartilage damage. A review", in this same Journal, on promising new strategies in the treatment of cartilage defects. The established treatments such as osteochondral implants, bone marrow stimulation techniques and chondrogeneic cell implantations, besides advantages, have drawbacks that have led to seek new strategies such as scaffold materials. Matrix-associated chondrocyte implantation, hyaluronan-based scaffolds, tissue-engineered collagen matrices seeded with autologous chondrocytes and encapsulation of autologous chondrocytes in poly(ethylene glycol) diacrylate (PEGDA) seem to be less invasive and have a good performance. In this review we describe benefits and disadvantages of the new procedures of cartilage regeneration by scaffolding materials such as PEGDA.

Key words

Cartilage; tissue engineering; scaffold; PEGDA.

Introduction

Cartilage is a flexible connective tissue found in many areas of human and other animal bodies, including joints between bones. It is composed of specialized cells called chondroblasts and chondrocytes that produce a large amount of extracellular matrix composed of collagen fibers and abundant ground substance, rich in proteoglycan and elastin fibers (Musumeci, 2013a). According to the amount of these components cartilage is classified in three types: elastic cartilage, hyaline cartilage and fibrocartilage. Unlike other connective tissues, cartilage does not contain blood vessels. The cells that reside in the matrix are called chondrocytes, they are located in lacunae within the matrix (forming chondrons together with the pericellular matrix) and represent only 5% to 10% of the total cartilage volume but are crucial to the maintenance of a stable extra-cellular matrix (Musumeci, 2013b). The chondrocytes are supplied by diffusion, helped by the pumping action generated by compression

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of the articular cartilage or flexion of the elastic cartilage (Musumeci, et al., 2013c; Pichler et al., 2013).

Increasing the source of cells for artificial reparation of cartilage defects is becoming an issue. The limited supply of cartilage, as a source of chondrocytes, requires a phase of expansion in monolayer culture. Chondrocyte expansion is complicated by the fact that monolayer-cultured chondrocytes de-differentiate, lose their characteristic phenotype and synthesize type I rather than type II collagen (Rahfoth et al., 1998). This has led to investigation into the use of stem cells. Stem cells can be relatively easily harvested and the procedures using stem cells are less invasive or destructive than articular cartilage harvesting procedures. The inherent ability of stem cells to self-renew opens the possibility that cell expansion may be achievable post-implantation (Wang et al., 2003). The differentiation of stem cells into different cell types is reliant on the local microenvironment, and growth factors, extracellular matrix and mechanical forces are provided by bioreactors (Wang et al., 2003; Angele et al., 2004; Miyanishi et al., 2006). Bioreactors, which apply load, have been frequently used in chondrogenic studies. However, the aim of those studies has been to pre-condition the implant prior to implantation, mainly by axial loading, which does not reflect the complexity of joint motion. The appropriate use of a bioreactor can be to apply load, which more closely reproduces the *in vivo* joint kinematic and would better predict what would occur to an implant after implantation into a defect. Thus by specifically employing such a bioreactor system as an intermediate step prior to animal studies we can screen new repair techniques under stimulated *in vivo* conditions. In this review we describe benefits and disadvantages of the new procedures of cartilage regeneration by scaffolding materials such as PEGDA.

New Perspectives

In view of the problems and drawbacks inherent to all long established techniques (see accompanying review in this same issue) we will refer to the encouraging hypothesis, stemming out of different studies, that a novel method to improve chondrocytes delivery *in vivo*, their proliferation and cartilage regeneration may be represented by encapsulation of autologous chondrocytes in poly(ethylene glycol) diacrylate (PEGDA) (Figs 1, 2, 3) to protect the cells from the harsh environment of inflamed joints. This method increases the survival rate of chondrocytes while they are slowly being released from encapsulation and incorporated into new cartilage tissue.

First of all, chondrocytes should come from mesenchymal stem cells (Fig. 4), which differentiate in chondrocyte progenitors. Use of mesenchymal stem cells provides a renewable source of chondrocytes. It is necessary a minimally-to-non invasive method to isolate mesenchymal stem cells from patient bone marrow or from adipose tissue (Musumeci et al., 2011a). Mesenchymal stem cells, *in vitro,* need to be cultured, to grow and differentiate into chondrocytes. After their isolation, the mesenchymal stem cells are expanded on a matrix of polyethylene oxide, chitin and chitosan in standard culture medium. Optimized levels of chondrogenesis have been achieved using a scaffold composed of 30% polyethylene oxide, 20% chitin and 40% chitosan (Kuo and Ku, 2008). This step is necessary because cartilage repair techniques require a certain amount of healthy native cartilage (Wright, 2009). In order

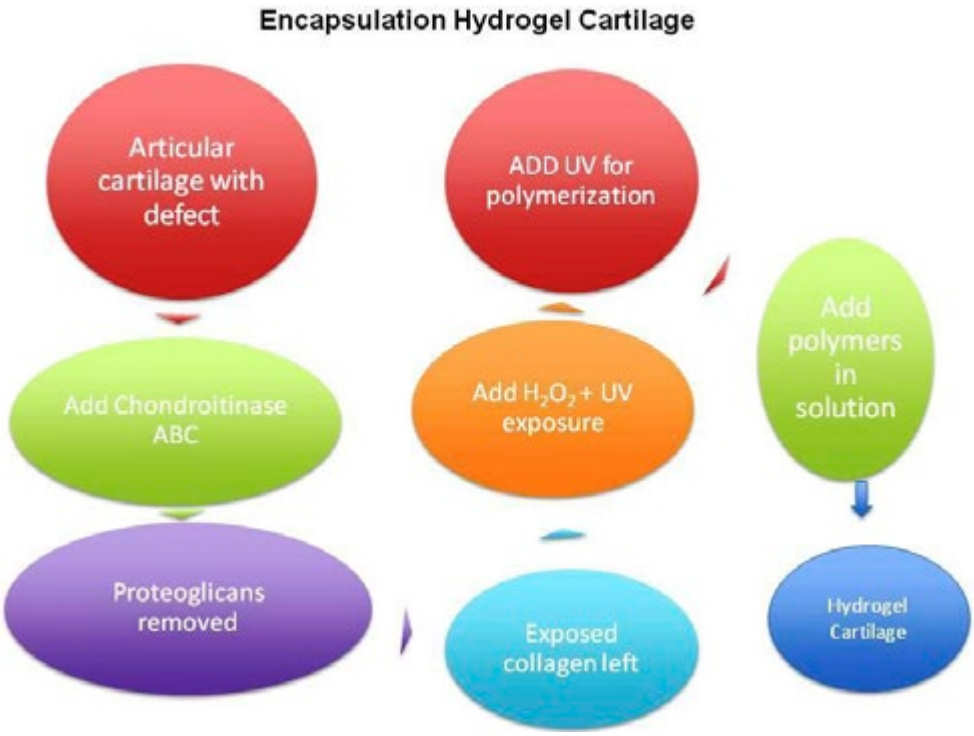

Figure 1 – Diagram of encapsulation of cartilage cells in hydrogel.

to differentiate the mesenchymal stem cells into chondrocytes, they are pretreated with transforming growth factor-β1 (TGF- β 1), necessary for chondrocyte differentiation, followed by insulin-like growth factor-1 (IGF-1) that increases extra-cellular matrix production and deposition by the cells (Worster et al., 2001). Cultured chondrocytes can be harvested from the polyethylene oxide/chitin/chitosan matrix using an established procedure involving incubation with collagenase and fetal bovine serum (Musumeci et al., 2011b). Chondrocytes derived from these mesenchymal stem cells should be encapsulated and characterized. The harvested chondrocytes are then suspended in a PEGDA solution in sterile PBS with penicillin and streptomycin. After encapsulation of autologous chondrocytes in PEGDA, *in vitro* tests are required to verify if these cells are able to survive better than non-encapsulated cells when exposed to inflammatory conditions similar to those found in a degenerating articular cartilage. After injury, cell apoptosis and inflammation lead to a very toxic local environment that adversely affects cell proliferation and survival. By encapsulating these chondrocytes, the cells are better able to resist the harsher environment. While *in vitro* encapsulation promotes survival, the *in vivo* and long-term efficacy of this treatment is unknown, given the different chemical and mechanical loading conditions. It is also unknown if matrix remodeling is able to clear the PEGDA from the wound area once the chondrocytes have proliferated to an acceptable degree.

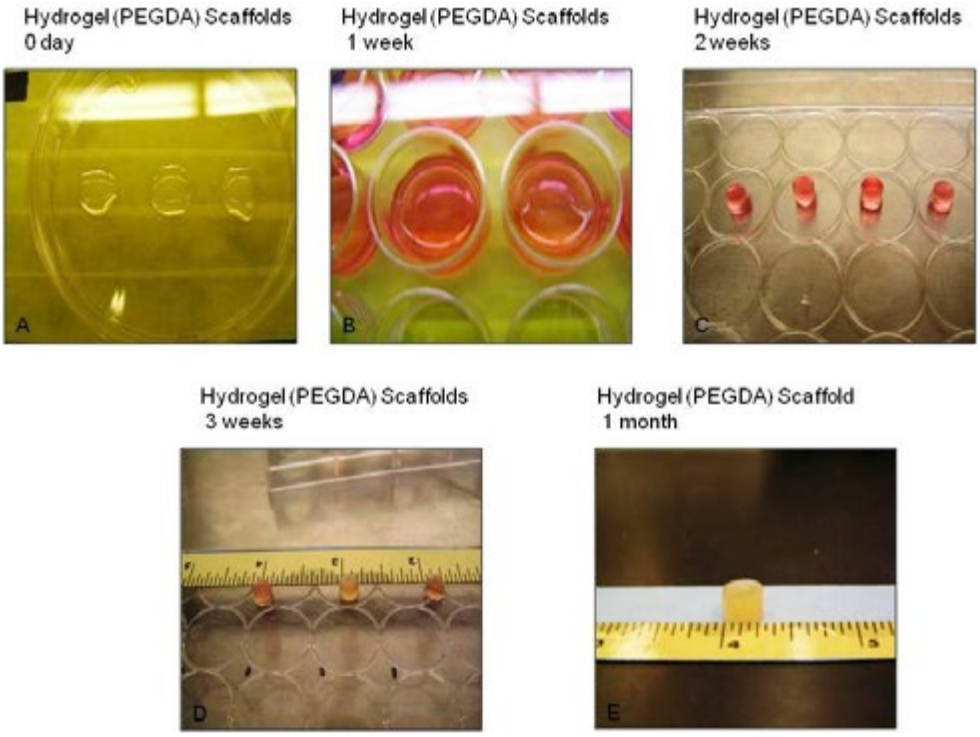

Figure 2 – In this figure the chondrocytes into the Hydrogel PEGDA scaffold are shown since 0 day to 1 month culture. Notice the difference in size between the hydrogel scaffold at 3 weeks of encapsulation and after 1 month. This suggests that there is cellular growth in the hydrogel as confirmed by histology (increase in proliferation, cellular aggregations in nests, typical structures of the hyaline cartilage, and an increase in ECM production), described in details in our recent study (Musumeci et al., 2011b). A) day 0. B) 1 week. C) 2 weeks. D) 3 weeks. E) 1 month.

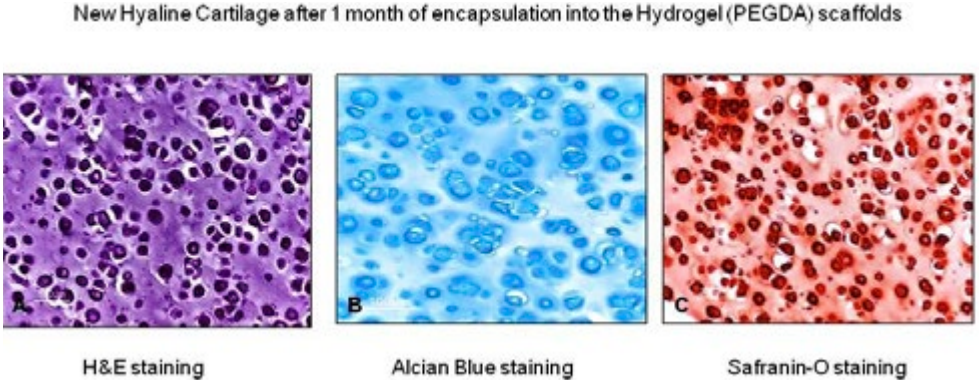

Figure 3 – New hyaline cartilage after 1 month encapsulation in a hydrogel (PEGDA) scaffold. (A) Haematoxylin and eosin. (B) Alcian blue. (C) Safranin-O. Original magnification x20.

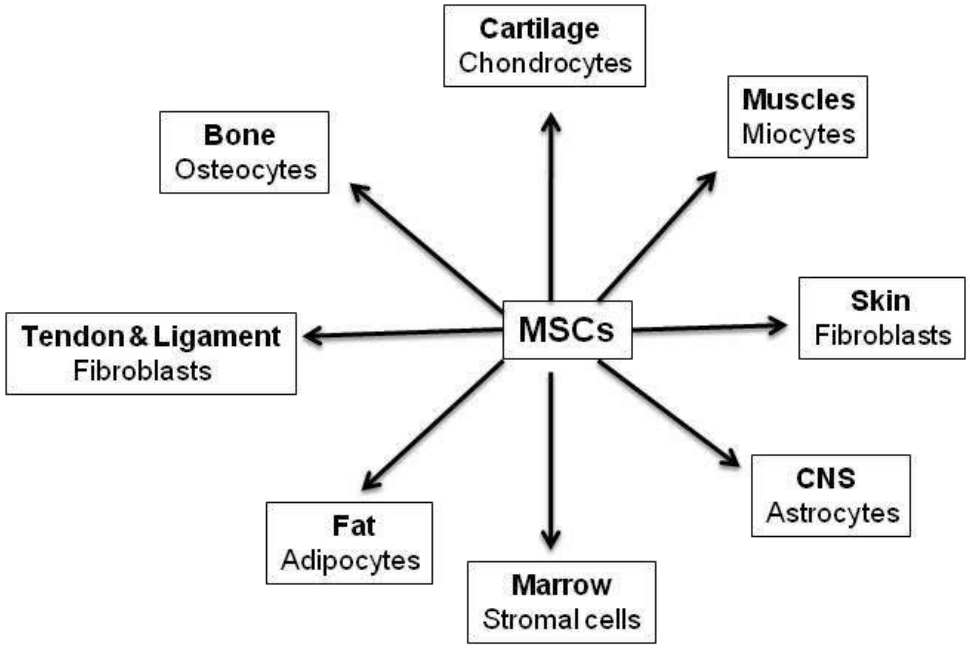

Figure 4 – Diagram of possible differentiation pathways of bone marrow derived mesenchymal stem cells (MSCs).

The feasibility of long term *in vivo* survivability and effectiveness should be further investigated. Check tests to extrapolate properties of the tissue *in vivo* are necessary, so it is fundamental to evaluate some parameters such as measuring cell viability, apoptosis (Musumeci et al., 2011b), β-Defensin-4 (Musumeci et al., 2012), lubricin (Musumeci et al., 2011c), mechanical properties (Julkunen et al., 2008) and biochemical composition (Suh and Matthew, 2000). Also, histological analysis is required to assess the health of the implanted tissues, encapsulated versus non-encapsulated, and additional tests may be applied to check for the degradation of PEGDA encapsulation (Musumeci et al., 2011c). At last, it remains to check the long-term survival of the product. The advantages of this treatment method consist in its relative flexibility and minimally invasive nature. Furthermore, the time length of about two months is acceptable clinically. The drawbacks of this treatment are that it may not be successful for an autoimmune disorder like rheumatoid arthritis and that there is not yet much evidence to suggest that the encapsulated cells have the same mechanical properties of native chondrocytes. It is not possible to predict if the patient will regain full motion and quality of life. However recent data support this new technique (Musumeci et al., 2011c; Musumeci et al., 2011b), and results of these studies suggest the possibility of applying autologous cell transplantation in conjunction with scaffold materials for repairing cartilage lesions in patients with osteoarthritis to reduce at least the progression of the disease.

Conclusion

Cell-based techniques performed with or without a scaffold have demonstrated good results in animal and basic-science models such as bioreactor, but further in vivo and in vitro studies must be carried out in order to confirm their successful clinical outcomes.

Acknowledgments

The authors would like to thank Prof. Jennifer Elisseeff, Department of Biomedical Engineering and Orthopedic Surgery, Johns Hopkins University of Baltimore, Maryland, USA, Prof. Mauro Alini from the AO Research Institute, Davos Platz, Switzerland, for her valuable help, and the undergraduate Bioengineering students from Berkeley, University of California, Alisha Manandhar, Hawnlay Swen, Stephen Trisno, Tiffany Truong, for the idea and important collaborations.

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