Collagen-Derived Biomaterials in Bone and Cartilage Repair

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Summary: We have analyzed a number of collagen-derived biomaterials for the matrix- induced and assisted bone and cartilage tissue regeneration. These include the Small intestine submuosa (SIS) Restor ™, ACI-Maix collagen membrane, Chondro-Gide collagen membrane, Permacol collagen Ossix and lycoll collagen membrane and five types of collagen-based marine sponge skeletons. Certain characteristics of different scaffold materials with comparable chemical composition may vary significantly. This variation may have a relevant impact on the suitability of the scaffolds for bone and cartilage regeneration. It suggests that the ACI-Maix® membrane is the best available collagen-derived material for an MACI®/MACT® application. In addition, the study of marine sponge indicates that the collagenous fibre skeleton of marine sponges provides a suitable bioscaffold for bone regeneration, as it supports the adhesion, migration and proliferation of osteoblasts *in vitro*.

Keywords: biomaterials; collagen membrane; cell therapy; MACI; marine sponge

Introduction

A key aspect of cell-based tissue engineering for tissue repair and regeneration is the use of suitable scaffold biomaterials. Ideally, scaffold materials should be a functional and structural biomimetic of the native extracellular matrix of the tissue and are capable undergo degradation when the functional tissue is formed.[2] Although a wide variety of scaffold materials are available, it is important in the field of regenerative medicine to select a material that closely matches the properties of the tissue it seeks to replace.[3] Each type of material is associated with a specific and unique host response when implanted, therefore the biocompatibility of the scaffold material also needs to be considered.[1] Identification of scaffolds onto which osteoblast or chondrocytes can be seeded to generate functional three-dimensional bone and cartilage tissues is one of the major approaches in orthopaedic tissue Engineering. For example, matrix induced autologus chondroyte implantation (MACI) utilizes type I/III collagen membrane for the regeneration of cartilage within the osteochondral lesions.

Over the past decade, a number of collagen-derived biomaterials have been developed for matrix-induced and assisted bone, cartilage and tendon tissue regeneration. These include the Small intestine submuosa (SIS) Restor ™, ACI-Maix collagen membrane, Chondro-Gide collagen membrane, Permacol collagen Ossix and lycoll collagen membrane. In addition, collage-based marine sponge skeletons have also been used as a potential collagen biomaterial for bone repair. In this study, we have conducted biochemical and pre-clinical evaluation on the use of these collagen materials for autologous chondrocyte implantation. We have also investigated the feasibility of marine sponges as a scaffold for bone regeneration.

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Materials and Methods

Collagen-derived Biomaterials for Cartilage Repair of MACI

The following commercially available collagen membranes were tested before and after standardised loading with human chondr ocytes: Chondro-Gide® (Geistlich, Switzerland), ACI-Maix® (Matricel, Germany), Ossix® (Colbar, Israel), Lycoll® (Resorba, Germany) and SIS (DePuy, US). Human chondrocytes were isolated from cartilage samples and grown in a standardised way. For seeding of the biomaterials samples ($1{,}0 \times 1{,}0$ cm) were placed in 12-well plate, fixed with a Teflon ring and loaded with 1.0×10^5 cells. All experiments were run in six fold and repeated at least 3 to 5 times to ensure statistical significance. Scanning electron microscopy of cell-free scaffold materials was performed according to standard protocols. Histological staining with HE was performed on paraffin embedded slides of seeded biomaterials. The biocompatibility of the membranes was investigated via culturing chondrocytes on the material for 3 days. Following enzymatic digestion of the carrier material the cells were harvested by centrifugation and the total cell number and viability were determined by Trypan blue staining. Tearing strength of the two membranes was analysed with a special device: Membrane samples of 1 cm^2 were fixed in metal holders and the force required to tear the sample was recorded by a tonometer.

Collagen-derived Biomaterials for Bone Repair

The availability of suitable scaffolds for the treatment of bone-related defects is limited. Marine sponge skeletons were therefore selected for use as bioscaffolds for bone repair on the basis of their collagen fiber extracellular matrix, interconnecting canal systems forming porosity, ability to hydrate to a high degree, and the diverse skeletal architecture within the phylum Porifera. Five unidentified sponge species of the genus *Hippospongia* (1), the genus *Callyspongia* (3), and the family *Chalinidae* (1), where selected, decellularized and seeded with osteoblasts. Using a combination of light, confocal and scanning electron microscopy miscopy described above, the sponge skeletons were assessed for potential use as a bioscaffolds for bone- related defects.

Results

Collagen-derived Biomaterials for Cartilage Repair

Under the SEM, all membranes show a structure with a dense and a porous side. They differ in density and pore size [Figure 1]. Chondro-Gide® and ACI-Maix® are structured similarly with a network of collagen fibres on the upper ''rough'' surface and a thin and denser ''smooth'' layer to form a bilayered construct. The cross section of Lycoll® membrane demonstrates a similar structure with a homogenous distribution of pores. SIS and Ossix® are more dense and smooth with a less complex interior structure. Histological section of chondrocytes seeded collagen materials showed the distribution of cells within the biomaterials was investigated. The sections of Chondro-Gide®, ACI-Maix® and Lycoll® demonstrate the attachment of the cells to the surface as well as their integration into the material [Figure 1B]. On Ossix® and SIS cell layers are stained mainly in the superficial zone of the biomaterial. A significant number of cells could also be detected in deeper layers of SIS. Sections of blank SIS membranes also showed the presence of cell material in deep layers. Probably the SIS membrane contains cell artefacts deriving from the original material. Next, the cell viability assay demonstrated high viabilities between 95 and 98% for all investigated membranes. The mean cell numbers that were obtained after three days of incubation are comparable. SIS showed a higher yield of cells. The mechanical properties of the collagen materials was examined using a tear resistance analysis [Figure 4]. The results showed that the tear resistance is very similar from the investigation of

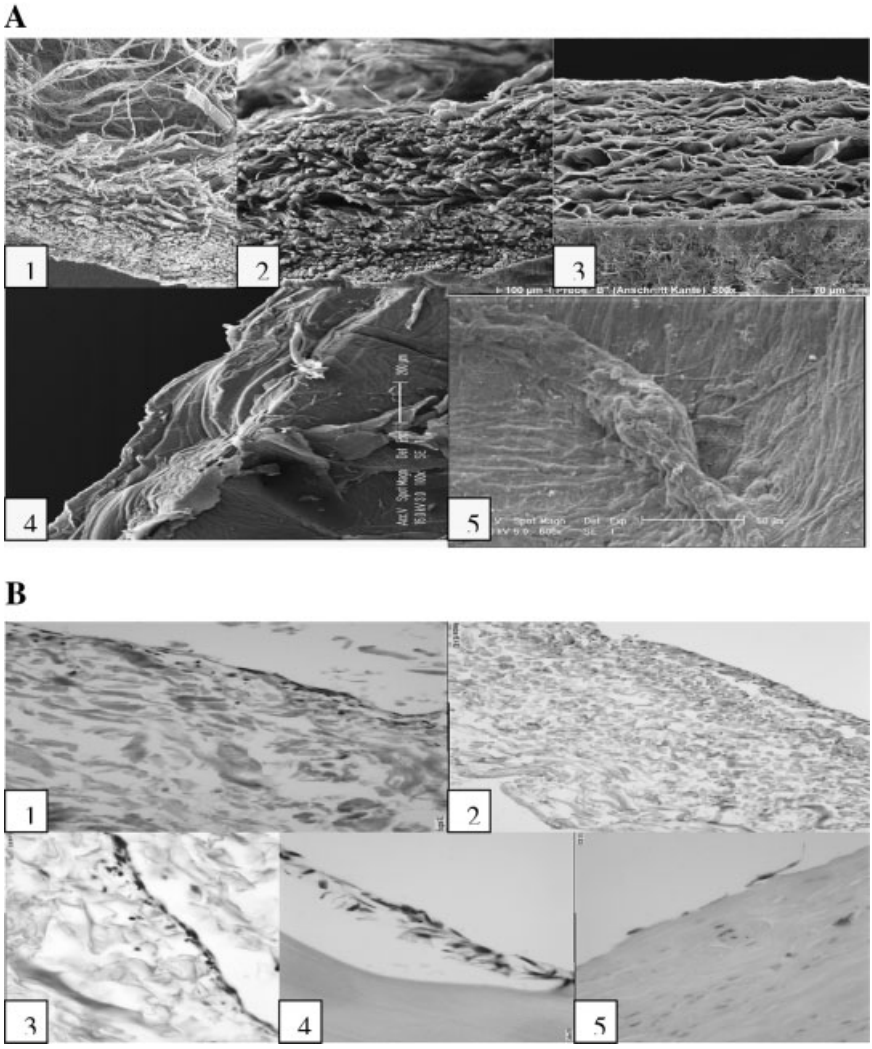

Figure 1.
Morphological observation of Collagen membrane (A) and cell inoculation (B). 1. Chondro-Gide®; 2. ACI-Maix®; 3. Lycoll®; 4.Ossix®; 5. SIS. Note that all membranes have different density and porosity (A) cells enable to attach to the surface of membrane as evidenced by H & E staining (B).

ACI-Maix®, Chondro-Gide and Ossix® in wet and in dry testing. SIS and Lycoll® differed significantly from this group: SIS showed an increased stability both in dry and in wet condition, while very low values were obtained for the Lycoll® membrane.

Osteoblasts isolated from mouse calvarias were seeded on various marine collagen sponges after subjected to Gamma irradiation. After 4 to 7 days co-culture, cell attachment was observed on all 5 species with cells aligning along the longitudinal axis of sponge fibres. By day 14 increased cellular invasion and proliferation was evident, with osteoblastic cells completely bridging interconnecting spongin-fibre pores at 21-days culture. Histochemical staining and scanning-electron microscopy studies confirmed the proliferating cells maintained their osteoblastic phenotype as evidenced by positivity for alkaline phosphatase and the ability to secrete extracellular matrix.

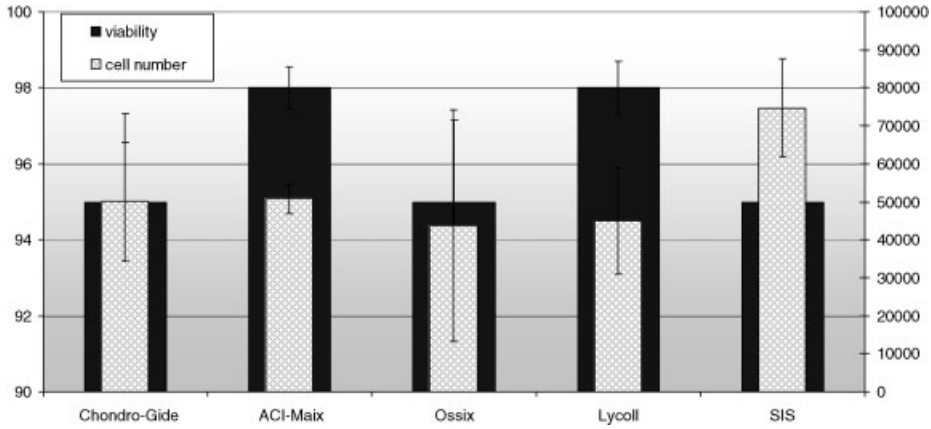

Figure 2.
Viability and cell number on the collagen membrane. Note that both ACI-Maix and Lycoll collagne membrane have better cell attachment and viability as compared to other membranes ($P < 0.01$).

Discussion and Conclusion

Although a wide variety of scaffold materials are available, it is important in tissue engineering to select a material that closely matches the properties of the tissue it seeks to replace.[3] Each type of material is associated with a specific and unique host response when implanted; therefore the biocompatibility of the scaffold material also needs to be considered (Atala & Lanza, 2002). We have compared the collagen derived membranes for cartilage repair of MACI and analysed the feasibility on the use of marine sponge as collagen scaffolds for bone repair.

All membranes showed a comparable basic structure comprising a smooth and a rough surface. Some of the detected morphological differences between the analysed membranes, however, may result in significant advantages in the use of these scaffolds in tissue engineering. The use of cell-seeded collagen membranes in the operating theatre requires certain stability and tear resistance. On the other hand the

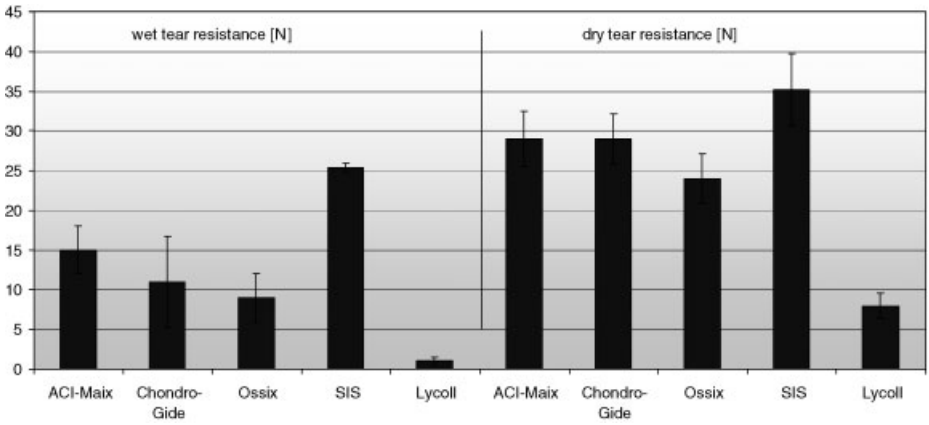

Figure 3.
Mechanical property of Collagen membrane (tear resistance). Note that SIS membrane has a best tear resistance property followed by the ACI-maix collagen membrane.

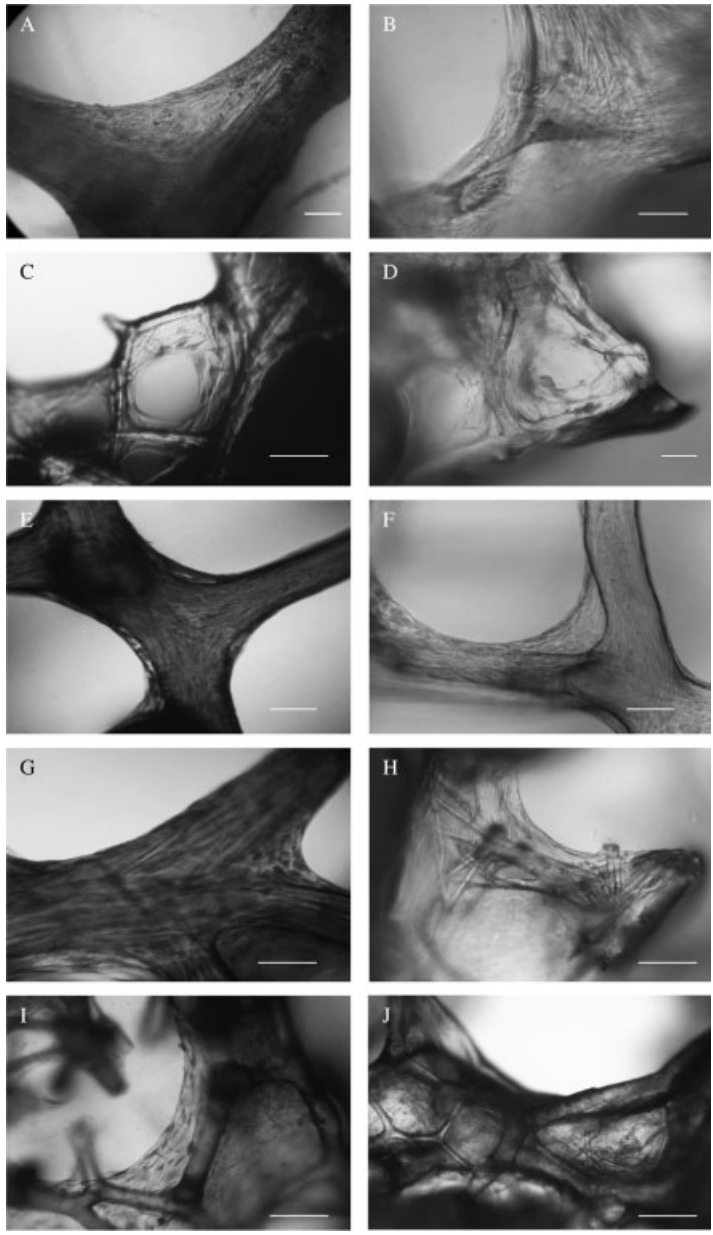

Figure 4.
Light micrographs of sponge-cell constructs after 7days *in vitro* culture. **Species 1,** (A) stained with Haemotoxylin and Eosin (H+E) showing cellular bridging between connecting fibres, and (B) stained for Alkaline phosphatase (ALP) activity, showing the osteoblastic phenotype of some cells (red); **Species 2,** (C) stained with H+E showing infiltration of a pore, and (D) stained for ALP activity; **Species 3,** (E) stained with H+E showing a layer of cells over the fibres, and (F) stained for ALP showing only slight activity; **Species 4**, (G) stained with H+E showing cell bridge interconnecting fibres, and (H) stained for ALP showing limited activity; **Species 5**, (I) stained with H+E showing cell coverage of pores, and (J) stained for ALP showing significant activity. Scale bar = 40 μm.

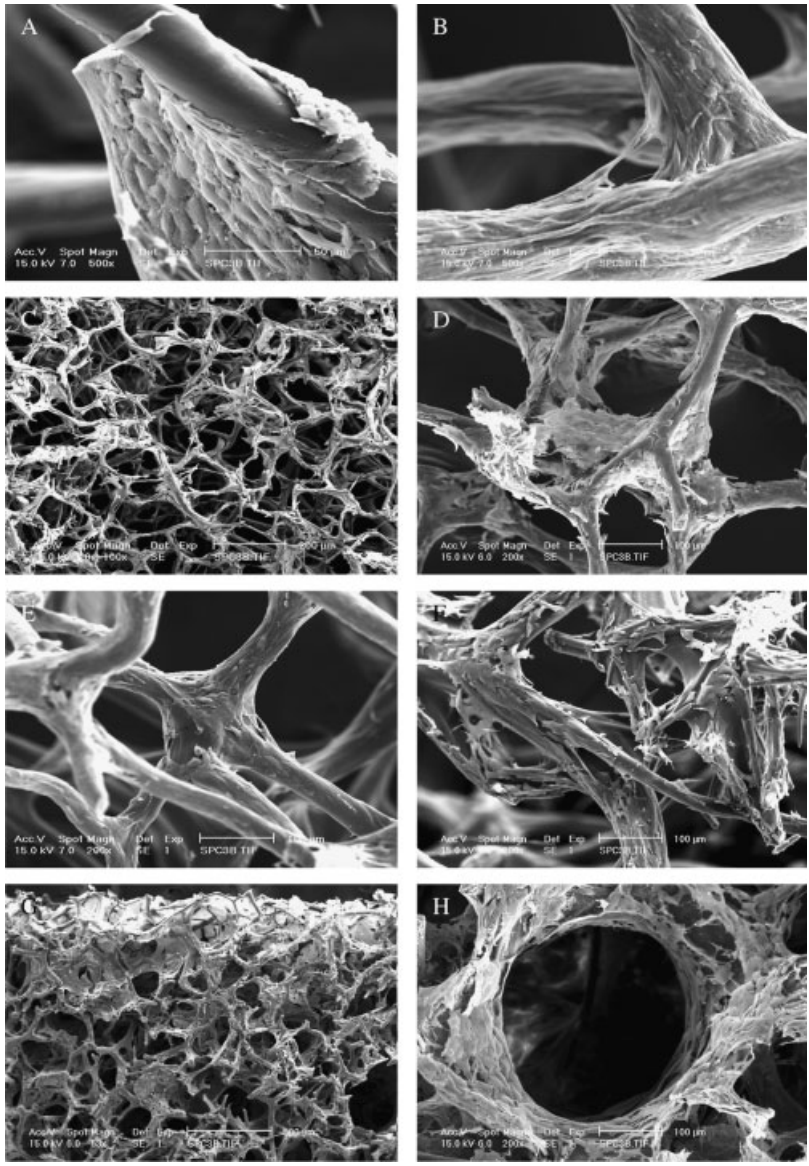

Figure 5.
SEM micrographs of sponge-cell constructs after 7 days culture. (A & B) **Species 1**, showing cells with globular and spindle-like morphology; **Species 2** at low (C) and high (D) magnification showing heterologous cell coverage; (E) **Species 3**, showing cells forming layers over the fibres of the sponge skeleton; (F) **Species 4**, showing sporadic cell growth over fibres and spicules; **Species 5** at low (G) and high (H) magnification, showing limited filling-in of large pores by cells.

carrier material should be flexible enough to be modelled into the cartilage defect and also have a reasonable resorption time. At the same time the environment for cell attachment, growth and differentiation needs to be optimal. A high microscopic surface with a larger space for cells to attach and penetrate into the scaffold material seems to provide for better cell adhesion and higher viability. This requirement,

however, has to be matched by a certain mechanical resistance. In respect to these investigations the optimal combination of features for a specific application has to be identified. It suggest that the ACI-Maix® membrane is the best available collagen-derived material for an MACI®/MACT® application.[4]

The study of marine sponge indicates that the collagenous fiber skeleton of marine sponges provides a suitable bioscaffold for bone regeneration, as it supports the adhesion, migration and proliferation of osteoblasts *in vitro*. The abundance, availability, and structural diversity of natural marine sponge skeletons indicate a promising new source of scaffold for bone regeneration. However, further study is required to validate the biocompatibility and the osteoinductivity of the marine sponge.

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